

**The Role of Hearing in the Brown Anole (*Anolis sagrei*,
Polychrotidae): A Behavioral Perspective**

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DEDICATION

I dedicate this dissertation to my mom, Janet A. Cantwell, dad, Cameron W. Cantwell, and sister, Tara L. Cantwell, for their continued love and support throughout my life and teaching me to never give up, to Mac, my best friend and partner in life, for being patient and sticking with me through this journey, and to Roxy, the best companion and study pal a girl could have.



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ABSTRACT

All animals are likely to encounter a predator during their lifetime. Prey can reduce their risk of predation by recognizing dangerous situations and modifying their behavior accordingly. Many animals are known to utilize auditory predator cues to assess risk. However, lizards have historically been assumed to emphasize vision to assess risk because most species do not vocalize and, therefore, do not themselves communicate using sound. I conducted a field experiment to investigate the ability of the brown anole (*Anolis sagrei*, Polychrotidae) to 1) use auditory cues to evaluate predation risk and 2) distinguish between threatening and non-threatening avian calls (Chapter I). Next, I explored the importance of sound pressure level of auditory cues to assess the proximity of a predator under natural conditions (Chapter II). Finally, using a laboratory experiment, I compared lizard responses to the calls of a known sympatric predator and three distantly related unknown allopatric predators to further understand the role of familiarity with calls or similarity in call characteristics in driving antipredator behavior (Chapter III). My results indicate that brown anoles not only utilize auditory cues to assess predation risk, but also that familiarity with cues may be an important factor in auditory predator discrimination. My research provides a greater understanding into the sensory mechanisms these lizards use to assess their surrounding environment and, importantly, provides a greater understanding into the proximate mechanisms by which animals recognize auditory predator cues.

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INTRODUCTION

All organisms acquire information about their surroundings through sensory systems that function to receive and process stimuli which may subsequently be used to adjust to their surroundings. Information provided by stimuli may facilitate the maintenance of homeostasis, synchronization between timing and activities (e.g. breeding season), locating of threats or resources (e.g. food, mates and suitable habitats), or it may function to modify the behavior of the intended receiver as in communication between conspecifics or heterospecifics (Bradbury and Vehrencamp 2011; Dusenbury 1992). Stimuli assume two forms: cues (i.e. some property associated with a particular condition of interest) and signals (i.e. a stimulus produced by one individual that is intended for another individual. Signals are assumed to result in an average net benefit for both individuals). They may be transmitted and received using various modalities (e.g., tactile (touch), visual (light), olfactory (chemical), and acoustic (sound)) (Bradbury and Vehrencamp 2011).

A sense of hearing offers animals the opportunity to utilize sound to acquire information about their surrounding environment. It has evolved in both invertebrates (Stumpner and von Helversen 2001) and vertebrates (Fay and Popper 2000) and is primarily used for perception, localization and discrimination of sounds (Dooling and Popper 2000). Sounds may be used both as a means of communication and/or to enhance an organism's ability to detect, locate and recognize potential mates, prey, or threats (Stumpner and von Helversen 2001; Wever 1978).

This dissertation deals with the extent to which an animal species utilizes sound in the recognition of potential threats. Since most animals experience some sort of threat during their lifetime (in particular from predators) selection should favor the use of sensory cues that enable

them to identify potential dangers and respond in a manner and intensity associated with the level of risk (Bradbury and Vehrencamp 2011). For example, predator vocalizations and alarm calls provide prey with important information about predation risk including (but not limited to): predator type, proximity and/or location (Rosier and Langkilde 2011), and level of threat associated with the predator (Magrath et al. 2015; Templeton et al. 2005). Because predation accounts for a large percentage of mortality in animals, the above information ensures that prey are able to recognize and respond appropriately to a threat, thereby increasing its chances of survival (Caro 2005; Lima and Dill 1990).

Despite the fact that most predators typically do not vocalize when hunting, empirical evidence suggests that birds (Adams et al. 2006; Billings et al. 2015; Zanette et al. 2011), rodents (Order: Rodentia; Blumstein et al. 2008; Kindermann et al. 2009), fish (Remage-Healey et al. 2006), amphibians (Llusia et al. 2010), bats (Baxter et al. 2006), elephants (Thuppil and Coss 2013), and primates (Gil-da-Costa et al. 2003; Macedonia and Yount 1991), show an antipredatory response to the sounds produced by potential predators (see also review by Hettena et al. 2014). Caro (2005) and Hettena et al. (2014) address the extent to which the ability to recognize and respond to vocalizations by a particular predator may be innate versus require recent experience with a particular predator. Further, the mechanism underlying predator recognition can vary among species or populations of a species that might be exposed to different predators (Caro 2005; Fallow et al. 2013).

Non-avian reptiles of the Order: Squamata have a highly sophisticated and sensitive auditory system (Dooling et al. 2000; Wever 1978). Yet few studies have explored the role of hearing in relation to predator-prey interactions. Squamates are traditionally classified into two clades, Iguania and Scleroglossa (Pianka and Vitt 2003; although see Vidal and Hedges 2005,

2009). Iguanians, including agamids, chameleonids and iguanids, are characterized by having a fleshy tongue, primarily utilized for feeding. In addition, non-herbivorous iguanians utilize a sit and wait foraging mode where they ambush their prey, therefore they rely heavily on visual cues to detect and discriminate prey. In contrast, scleroglossans, including geckonids, skinks, monitors and many others, are mostly active foragers that typically use their powerful jaws for subduing prey and feeding, releasing their hard (keratinized) tongues for chemoreception. Iguanians and scleroglossans exhibit remarkable behavioral and ecological differences, which may have been driven by the acquisition of chemosensory abilities in scleroglossans (Pianka and Vitt 2003; Vidal and Hedges 2005, 2009).

While the functional morphology of the ear and the neurophysiology of the auditory system of lizards have been relatively well-documented, their use of hearing as an antipredatory defense has received little attention (but see Cantwell and Forrest 2013; Hettner et al. 2014; Ito and Mori 2010; Vitousek et al. 2007). A behavioral perspective is largely lacking. In this study, I examined the antipredatory response of an anole to the vocalizations of its predators. Lizards of the genus *Anolis* (anoles, Polychrotidae, Anolis, although see Nicholson et al. 2012) are small to medium-sized lizards (33-191 mm SVL) that vary in size, color and pattern. Anoles are distinguished by their dewlap, a throat extension, which is used in territorial and courtship displays. These lizards exhibit color vision and exceptional eyesight and are, therefore, considered highly visually oriented (Losos 2009).

Most behavioral studies reported on anoles to date have focused on vision and few studies have investigated the role of hearing in ecological settings. Here, I report the results of studies designed to assess the ability of brown anoles (*Anolis sagrei*) to 1) recognize and respond to predatory and non-predatory avian vocalizations (See Chapter 1), 2) distinguish the call of

bird species known to prey on lizards from the call of species which rarely, if ever, do so (See Chapter 1), and 3) use auditory distance cues to determine the proximity of an avian predator (See Chapter 2). Finally, Chapter 3 compares the ability to identify the underlying mechanisms that assist in discrimination of sympatric and allopatric saurophagous avian predators which are also phylogenetically distinct from one another.

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**CHAPTER I: A COMPARATIVE STUDY OF THE BEHAVIORAL RESPONSES OF
BROWN ANOLES TO AUDITORY PREDATOR CUES – SKITTISH FEMALES
AND DISCRIMINATORY MALES**

Abstract

Predator recognition enables prey to accurately and efficiently respond to various cues and signals within a given environment. Prey that can recognize auditory predator vocalizations are able gain information about current predation risk associated with the proximity and identity of a predator. While the ability to recognize and respond to auditory predator vocalizations is well documented in birds and mammals, less is known about lizards' ability to acquire information about predation risk from auditory cues. We investigated the ability of wild male and female brown anoles to discriminate between the vocalizations of a sympatric predator (American kestrel), a sympatric non-predator (Northern cardinal), and white noise. Male anoles responded in a manner consistent with the threat-sensitivity hypothesis; males exhibited the greatest vigilance in response to a playback of the American kestrel call compared to the call of a Northern cardinal and white noise and were also more vigilant during the Northern cardinal call compared to white noise. In contrast, female anoles were more vigilant than males during both baseline observations and playbacks of auditory cues, in general. Our results, combined with recent research, points to the importance of learning for male anoles to recognize and respond to predatory kestrel vocalizations and highlights the difference in responses between male and female lizards.

Introduction

During a predatory encounter an animal that fails to respond with antipredator behavior or responds inappropriately is likely to suffer high fitness consequences. However, responding with antipredator behavior during a nonthreatening situation can be energetically costly or result in the loss of time searching for food or mates (Ydenberg and Dill 1986). Efficient antipredator behavior can minimize these costs; as a result, selection favors prey that are able to acquire and use information to accurately assess predation risk and modify their behavior accordingly (Lima and Dill 1990). One mechanism that aids prey in risk assessment is predator recognition, the ability to distinguish threatening from non-threatening or less dangerous cues (Caro 2005). Prey may possess the ability to recognize predators immediately after birth (i.e. innate predator recognition) or they may require some level of experience to recognize the predator (Caro 2005; Hettner et al. 2014). Moreover, animals may use a variety of sensory modalities (e.g. olfactory, visual, and auditory) to reliably detect the presence of a predator and discriminate between dangerous and nonthreatening stimuli.

Auditory cues have the potential to alert an individual to the presence of a predator earlier than visual cues and, unlike chemical cues, do not linger in the environment once the predator has moved to a new location; therefore auditory cues provide information consistent with the predator being in the area at present (Bradbury and Vehrencamp 2011; Weissburg et al. 2014). Additionally, auditory cues, such as predator vocalizations and alarm calls, can provide prey with information about the location, proximity, or identity of a predator (Rosier and Langkilde 2011); therefore, eavesdropping on auditory cues is beneficial because it decreases environmental uncertainty associated with predation risk (Dall et al. 2005; Magrath et al. 2015; Ridley et al. 2014). Empirical evidence corroborates the reliability of auditory cues in providing prey with

information about the presence of a predator (Blumstein et al. 2008; Hettena et al. 2014). Responses to auditory cues include increased vigilance (Adams et al. 2006; Blumstein et al. 2008; Saunders et al. 2013), escape (Thuppil and Coss 2013) and cessation or reduction of activity (Blumstein et al. 2008; Huang et al. 2011; Ramage-Healey et al. 2006). Furthermore, the strength of the antipredator response may depend on the perceived risk associated with the auditory cue, as proposed by the threat sensitivity hypothesis (Helfman 1989). For example, playbacks of predator vocalizations (bald eagle calls and human voices) and heterospecific and conspecific alarm calls elicited a similar increase in vigilance in herring gulls (*Laridae: Larus* spp.) whereas great black-backed gulls (*Larus marinus*) responded most strongly to playbacks of predatory human voices followed by conspecific alarm calls and predatory bald eagle vocalizations (MacLean and Bonter 2013). In contrast, Barrera et al. (2011) found that zenaida doves (*Columbidae: Zenaida aurita*) exhibited a higher degree of responsiveness to predator vocalizations compared to conspecific alarm wing whistles (see Hingee and Magrath 2009 for evidence of wing whistles as an alarm signal).

The use of auditory cues to assess predation risk is well studied for birds and mammals (see review in Hettena et al. 2014). However, interest in the ability of lizards to acquire information about predation risk from auditory cues only piqued in the last decade. To date, lizards from five different families have been shown to recognize and respond to auditory cues associated with predation risk. For example, Vitousek et al. (2007), found that Galápagos marine iguanas (*Iguanidae: Amblyrhynchus cristatus*) eavesdrop on the alarm call of the Galápagos mockingbird (*Mimidae: Nesomimus parvulus*) and respond with antipredator behavior. Likewise, playbacks of heterospecific alarm calls elicit increased vigilance in the Madagascan spiny-tailed iguana (*Opluridae: Oplurus cuvieri cuvieri*) (Ito and Mori 2010) and induced quicker and darker

body color changes in the day gecko, *Phelsuma kochi* (Gekkonidae) (Ito et al. 2013).

Moreover, exposure to sympatric predator vocalizations resulted in decreased display rates in free-ranging crested anoles, *Anolis cristatellus* (Polychrotidae) (Huang et al. 2011) and in increased vigilance in captive wild-caught brown anoles *A. sagrei* (Cantwell and Forrest 2013). In comparison, white-bellied copper-striped skinks (Scincidae: *Emoia cyanura*) were more likely to respond with antipredator behavior to heterospecific alarm calls compared to sympatric predator vocalizations (Fuong et al. 2014). Indeed, these findings are interesting because most lizards are non-vocal and rely on other sensory modalities for communication.

The diet of many species of predatory birds commonly includes lizards (e.g. Cruz 1976; Martín and López 1996; McLaughlin and Roughgarden 1989; Poulin et al. 2001; Wunderle Jr. 1981). Specifically, anoles make up ~40% of the kestrel (*Falco sparverius*) diet on Puerto Rico (Wetmore 1916), ~78% of the kestrel diet on Jamaica (Cruz 1976), and 50-75% of the kestrel diet on the island of Anguilla, in the northern Lesser Antilles (McLaughlin and Roughgarden 1989). Consequently, avian predation on lizards has been implicated as an important selective pressure on lizard behavior (Blomberg and Shine 2000; Costantini et al. 2007; Martín and López 1996; Stuart-Fox et al. 2003).

The objective of our study was to investigate the use of auditory cues by free-ranging male and female brown anoles (*A. sagrei*) to assess predation risk. In addition, we sought to identify whether males and females differed in their responses to playbacks of vocalizations of predatory and non-predatory birds. We compared the behavioral responses of anoles to playbacks of vocalizations from 1) a sympatric predator, the American kestrel (Falconidae: *Falco sparverius*), 2) a sympatric non-predator, the northern cardinal (Cardinalidae: *Cardinalis cardinalis*), and 3) white noise. We hypothesized that male and female lizards would perceive

predator vocalizations more threatening than non-predator vocalizations and control stimuli as evidenced by: 1) an increased likelihood of responding with antipredator behavior (see Table 1 for descriptions of various antipredator behaviors), 2) decreased latency to respond to respond to predatory stimuli, 3) increased vigilance during and after playbacks, and 4) that male lizards would decrease the amount of time spent dewlapping, a common territorial defense/courtship behavior.

Materials and Methods

Study species

Anolis sagrei is native to Cuba and the Bahamas, and was introduced into the southeastern United States around the 1940s (Bell 1953; Oliver 1950). Male *Anolis* are diurnal, social animals that use conspicuous displays during courtship and territorial defense (Greenberg 1977; Jenssen 1977), which may increase their risk of predation by avian predators (Costantini et al. 2007; Stuart-Fox et al. 2003; Vazquez and Hilje 2015). Although most anoles are non-vocal and typically thought of as being visually oriented (Losos 2009), they have well-developed auditory systems with enhanced directionality (Christensen-Dalsgaard and Manley 2005, 2008). Anoles have wide hearing sensitivity, ranging from 1 – 7 kHz (Brittan-Powell et al. 2010), which overlaps the range of frequency (1 – 5 kHz) used by most birds to communicate (Dooling et al. 2000).

Auditory Stimuli

We used a call from the American kestrel (*Falco sparverius*) for the predator stimulus because its geographic range overlaps that of the brown anole in both their native habitat (Cruz 1976) and Florida (Giery 2013) where they are known to feed on anoles. We used a song from the northern cardinal for the non-predator stimulus, as its diet consists mostly of seeds and fruit,

and they are commonly found at the location where the study was conducted. Additionally, we chose these two species because we were interested in validating the findings of a controlled laboratory experiment that suggests that brown anoles increase vigilance in response to kestrel calls relative to the calls of non-predatory birds and white noise (Cantwell and Forrest 2013). We obtained calls from the Cornell Laboratory of Ornithology Macaulay Library of Animal Sounds and generated a control (white noise) stimulus using Adobe Audition 3.0 (Adobe Systems Inc., San Jose, CA, USA).

Audio clips were 20 s in duration and normalized to 95% RMS peak amplitude using WavePad Sound Editor software v 4.59 (NCH Software Inc., Greenwood Village, CO, USA). Playback stimuli were broadcast through a Saul Mineroff SME-AFS field speaker (Elmont, NY, USA; 5 watts RMS; 100-12,000 Hz) positioned on an adjustable light stand that was connected to a MacBook Pro (Apple Inc., Cupertino, CA, USA). The speaker was placed at a horizontal distance of ~ 5-6 m from the focal subject. Speaker height was adjusted for the perch height of the lizard such that a lizard less than ~ 1.3 m off the ground was tested at a speaker height of 1.52 m, a lizard between ~ 1.3 and 2.4 m off the ground was tested at a speaker height of 2.44 m and lizards that were perched higher than ~ 2.4 m were tested at a speaker height of 3.35 m. Stimuli were played at 90 dB, measured at 1 m from the sound source using a Galaxy Audio Checkmate SPL meter (model# CM-140; time weighting: fast; frequency weighting: A; ± 1.5 dB).

Experimental Design

We conducted playback experiments in St. Johns County, FL, USA (29° 53' N / 81° 18' W) during June and July of 2011 on 96 adult male brown anoles and during June and July of 2012 on 27 adult female brown anoles. We used a repeated measures, balanced block design where we

randomly assigned male anoles to one of six sequences of three stimuli (N = 16 each: 1. predator-control-non-predator; 2. predator-non-predator-control; 3. non-predator-predator-control; 4. non-predator-control-predator; 5. control-predator-non-predator; 6. control-non-predator-predator) and female anoles were randomly assigned to one of three sequences of stimuli (N = 9 each: 1. predator-control-non-predator; 2. control-non-predator-predator; 3. non-predator-predator-control). Each stimulus was separated by one minute of silence. We recorded the lizards' behavior with a Sony Handycam digital camcorder (Sony Electronics, Inc., San Diego, CA, USA; model# DCR-DVD610) that was placed ~ 5 m from focal subject. Playback experiments were conducted between 0900 and 1500 hrs when anoles are most active.

We located anoles visually and allowed them to acclimate to our presence for five minutes prior to beginning a playback experiment. We recorded lizards for one minute before and after a playback sequence during which time we stood motionless to avoid disturbing the lizards. To prevent habituation to stimuli, lizards that were tested on the same day were separated by at least 50 m and different lizards in similar localities were tested every third day. Lizards were caught post-playback and measured for snout-vent length (SVL; mm), tail length (TL; mm), and weight (W; g). If we were unable to capture a lizard after testing, we tied pink flagging tape around a branch on the tree and that tree was not tested again in order to prevent testing the same animal twice. After completion of each trial we recorded sex, ambient temperature at 1 m from the ground (AT; C), perch temperature at 1 cm from the perch (PT; C), minimum and maximum wind speed (WS; m/s), and perch height (PH; m).

Behavioral Observations

Videos were scored for three categories of behavior (courtship/territorial behavior, antipredator behavior, and locomotion; Table I-1). Behavior of each lizard was quantified for 1)

the one-minute periods prior to the playback sequence, 2) the 20-second period prior to the start of a playback sequence, 3) each of the 20-second stimuli, 4) the two one-minute silence periods during a playback sequence, and 5) the one-minute period post playback sequence. We recorded 1) the lizards' first response elicited by a stimulus (hereafter referred to as 'antipredator behavior'), 2) time to respond to the stimulus (hereafter referred to as 'latency to respond'), 3) whether the response was directed toward the speaker, 4) the total number of antipredator responses for each period of time (hereafter referred to as 'total vigilance'), and 5) the total duration and bout duration of courtship/territorial behaviors for each time period. Three observers, two of whom were blind to the stimuli, scored videos using JWatcher v 1.0 video (Blumstein and Daniel 2007; <http://www.jwatcher.ucla.edu/>) to minimize observer bias. Inter-observer reliability scores were calculated using Cohen's kappa (k); all scores were 'good' ($0.6 < k \leq 0.75$) or 'excellent' ($k > 0.75$) with a median 'excellent' score across all observers ($k = 0.857$; Kaufman and Rosenthal 2009).

Data Analysis

Although we conducted 123 total trials on 96 males and 27 females, we excluded from the data analysis any trials, or portion of a trial, during which we were unable to observe a lizard's behavior for longer than 20 seconds (see Results for sample sizes). All statistical analyses were conducted with R version 3.1.2 (R Core Team 2014). We used an information-theoretic (IT) approach to model selection and multimodel inference (Burnham et al. 2011) to assess the immediate and short-term effects of playback type (control, non-predator, and predator) on anole behavior. To account for non-independence of lizards' responses due to testing with multiple stimuli, we fit generalized linear mixed models (GLMM), with a random intercept for each individual, using the adaptive Gauss-Hermite quadrature approximation (Bolker et al. 2009). We

used the ‘lme4’ package (Bates et al. 2014) to run all GLMM’s. We used q-q plots to assess the fit of the residual error of each global model for normality.

During exploratory analysis, we found sex by playback type interactions for antipredator behavior, latency to respond, and total vigilance both during playback and for the one-minute period following playback. In addition, we found strong or moderate correlations between SVL and weight ($r = 0.916$), SVL and tail length ($r = 0.527$), and ambient temperature and perch temperature ($r = 0.618$). Adult male brown anoles are larger than adult females (Tokarz 1985); therefore, we used ‘sex’ rather than SVL, weight, and tail length, for all global models. We also found an interaction between playback sequence order and playback type, when investigating the effect of playback type on courtship/territorial behavior. Moreover, courtship/territorial displays (dewlap, head bob, and push up; Table I-1) are not always independent of one another or mutually exclusive (Jenssen 1977). We found that dewlap duration and push-up/head bob duration were moderately correlated ($r = 0.517$), therefore, we only investigated the effect of playback type on total dewlap duration for the one-minute period following stimulus playback. It is important to note that we only included male lizards that displayed during at least one of the time periods (before, during, or after playback); if a lizard never displayed, it was not included in the analysis.

In addition to the main effects (playback type, sex, and playback sequence order) and interaction terms (playback type*sex and playback type*playback sequence order), we also included perch temperature, average wind speed, and perch height as fixed factors, and individual as the random factor in all global models. Because brown anoles are ectothermic, environmental temperature could affect how or if a lizard responds to stimuli. We used perch temperature rather than ambient temperature because it should be a better predictor of the

lizards' body temperature (Huey and Webster 1976). Average wind speed could potentially affect sound propagation by increasing or decreasing the amplitude of the playback stimuli (Bradbury and Vehrencamp 2011; Ingård 1953). Similarly, perch height might also affect lizard response due to differences in playback amplitude resulting from the distance between the lizard and the sound source or because of interference with sound waves (e.g. refraction of sound waves from the ground or canopy cover) (Bradbury and Vehrencamp 2011; Marten and Marler 1977; Weissburg et al. 2014). We included playback sequence order due to the possibility of lizards habituating to the stimuli since stimuli were played at one-minute intervals (Thompson and Spencer 1966).

To assess immediate effects of playback type on lizard behavior we investigated the likelihood of lizards responding with antipredator behavior to each of the three stimuli. We classified their first response to the stimuli as either no antipredator behavior (lizards did not respond to the playback or responded with courtship/territorial behavior; see Table I-1) or antipredator behavior, and used a GLMM with binomially distributed errors. Next, we fit a GLMM using gamma errors (link = log) to evaluate the effect of playback type on latency to respond to each stimulus and a GLMM with Poisson distributed errors to compare total vigilance (the number of antipredator responses) between playback stimuli and to the 20-second period prior to the start of a playback sequence, which we considered to be “normal” behavior. To assess the short-term effects of playback type on lizard behavior we compared total vigilance for the one-minute period after hearing each stimulus to the one-minute period prior to the start of a playback sequence for which we also used a GLMM with Poisson distributed errors. Last, we fit a GLMM with gamma errors (link = log) to compare the duration of dewlap displays for the one-minute period following stimulus playback.

We based model selection on Akaike's Information Criterion corrected for small sample size (AICc) except for when we detected overdispersion in the global model, in which case we estimated the variance inflation factor, $\hat{c}$, using the 'c_hat' function in the *AICcmodavg* package (Mazerolle 2015) and used quasi-AICc (QAICc) for model selection (Mazerolle 2006; Richards 2008). We fit all possible models for each analysis using the 'dredge' function in the package *MuMIn* (Bartoń 2014). The traditional rule has been that models with $\Delta_i > 2$ are of little value for inference, however Anderson (2008) suggests that important information can be found in models with Δ_i between 2 and 4; therefore, we considered all models with $\Delta_i \leq 4$ relative to the best-fitting model as equivalent in explaining the variation in the response variable (Burnham et al. 2011). Next, we used various functions in the *AICcmodavg* package (Mazerolle 2015) to calculate the Akaike weights (w_i), evidence ratios (ER) and the model-averaged parameter estimates, unconditional standard errors, and unconditional 95% confidence intervals for all candidate models. We used the *multcomp* (Hothorn et al. 2008) and *lsmeans* (Lenth and Herv   2015) packages to conduct pairwise comparisons when multi-level predictor variables were significant. All pairwise comparisons were run from the 'best' model from the candidate set of models for all analyses. Confidence intervals (CI's) were adjusted for multiple comparisons using the Tukey method and effects were considered significant if it did not contain zero.

Our results should be considered with one caveat in mind: we only tested lizards with one exemplar of each species call, thereby committing simple pseudoreplication (Kroodsma et al. 2001). Our use of generalized linear mixed models remedied the issue of non-independence in our observations, yet the problem of extrapolation to the wider population remains (i.e. treatments were not replicated) (Millar and Anderson 2004). Therefore, generalizations about how the lizards would respond to kestrels and cardinals, relatively speaking, should be made

with caution. However, we believe we would have attained similar results had we used multiple exemplars for each species as was done in Cantwell and Forrest (2013) who used eight exemplars for each species.

Results

Overall Summary

The most common antipredator behavior observed in both male and female lizards was ‘head movement’ and the least common antipredator behavior was ‘escape’ (Table I-1). Seven males and one female ‘escaped’ during playback of the predator stimulus, while only one male lizard ‘escaped’ during the control stimulus and two male lizards ‘escaped’ during the non-predator stimulus. In addition, nine lizards ‘displayed’ during the control stimulus, seven ‘displayed’ during the non-predator stimulus and five ‘displayed’ during the predator stimulus.

Speaker position relative to the lizard was recorded for 301 observations (231 males, 70 females), however we were unable to ascertain the direction in which a lizard responded for 97 observations because lizards either did not respond (47 males, 11 females) to the stimulus or responded with a non-directional response (e.g. raised or lowered head, crouched, raised body; 26 males, 13 females). Conversely, of the 204 observations (158 males, 46 females) that we could ascertain the direction a lizard responded, ~ 67% of males and ~ 52% of females responded in the direction of the speaker compared to those that did not. Summary statistics of physical characteristics and environmental conditions during testing for male and female lizards can be found in Table I-2.

Immediate Effects

Latency to respond

Lizards that never responded to a stimulus playback were not included in this analysis. We identified 7 candidate models with stimulus type present in all models; WS and PH were present in five models, sex was present in three models, and PO was present in one model. The “best” model found to explain the variation in lizard response times to the different stimuli included stimulus type, WS, and PH as predictors of response time and had a probability (w_i) of 0.38 (Table I-3).

We found that lizards that were perched higher and experienced higher wind speeds during testing took a significantly longer time to respond to a stimulus. In contrast, sex and playback order did not significantly affect response times (Table I-4). On average, lizards responded most quickly to the predator stimulus followed by the non-predator stimulus; they took the longest to respond to the control stimulus (Table I-5; Fig. I-1). Specifically, lizards responded significantly faster to both the predator and non-predator stimuli compared to the control stimulus. However, there was not a significant difference in lizard response times between the predator and non-predator stimuli (Table I-6).

Antipredator behavior

In total, 88.9% of lizards responded to the predator stimulus, 82.7% lizards responded to the non-predator stimulus and 67.3% lizards responded to the control stimulus (Table I-7). The most common first response elicited by all stimuli was ‘head movement’. In contrast, only two male lizards initially reacted with ‘escape’, both in response to the predator stimulus. Similarly, only two male lizards exhibited ‘squirrel’, one in response to the non-predator stimulus and one in response to the predator stimulus.

We identified 15 candidate models that best explained whether or not lizards responded with antipredator behavior to each of the stimuli. Stimulus type and playback order (PO) were present in all candidate models, while wind speed (WS) was included in eight models, sex was included in seven models, perch height (PH) and perch temperature (PT) were in six models, and the stimulus by sex interaction was in one model. The “best” model had a probability (w_i) of 0.17 and included stimulus type, PO and WS as predictors of response (Table I-3).

Perch temperature, perch height, wind speed, sex, and the stimulus*sex interaction had little to no effect on whether or not lizards responded with antipredator behavior to the three stimuli (Table I-4). We found that lizards were significantly more likely to respond with antipredator behavior to both the predator and non-predator stimuli compared to the control stimulus; however, lizards were equally likely to respond with antipredator behavior to the non-predator stimulus as they were to the predator stimulus (Table I-6). In contrast, lizards were significantly less likely to respond with antipredator behavior to the last stimulus played (third) relative to when it was played first. There was a marginally non-significant difference in response between the second and third playback positions (Table I-6).

Total vigilance prior to and during stimulus playbacks

We identified four candidate models that best explained total vigilance during stimulus playback. All models contained context (20 s before and during stimuli playback), sex, the interaction between context and sex, WS, and PO; both PT and PH were present in two models. The “best” model included context, sex, the interaction between context and sex, WS, and PO as predictors of the number of AP responses during stimulus playback and had a probability (w_i) of 0.51 (Table I-3).

Summary statistics for male and female vigilance behavior for the 20-second period before and during playback stimuli can be found in Table I-8. We found that higher WS had a significant negative effect on responses and that responses decreased when a stimulus was played second or third compared to when they were played first (Table I-4). However, AP responses did not differ between the second and third playback position. PT and PH did not have a significant effect on AP responses (Table I-4). Moreover, we found that male lizards exhibited significantly more AP responses to predator and non-predator stimuli compared to the 20 s period prior to the start of a playback sequence (Before). In addition, male lizards exhibited significantly more AP responses during playback of the predator stimulus compared to the non-predator stimulus and the control stimulus. Male lizards also exhibited significantly more AP responses to the non-predator stimulus relative to the control. Conversely, male responses to the control stimulus did not differ from ‘Before’ stimulus playback (Figure I-2; Table I-6). Female AP responses to all stimuli were not different from ‘Before’, nor did their responses differ between stimuli (Figure I-2; see Table I-6 for all comparisons and 95% CI’s).

Short-term Effects

One-minute post-playback total vigilance

We found 3 candidate models to best explain the difference in the number of responses after hearing the various stimuli. The “best” model, which had a probability (w_i) of 0.58, contained context, sex, the context by sex interaction, and WS, which were also present in all three models. PT and PH were each present in one model (Table I-3).

Table I-8 presents the summary statistics for male and female vigilance behavior for the one-minute period before and after playback stimuli. We found that higher WS significantly decreased the number of AP responses; conversely, PT and PH did not have a significant effect

on responses (Table I-4). In contrast, male lizards exhibited significantly more AP responses after hearing the predator stimulus compared to the one-minute period before and after hearing both the control and the non-predator stimuli. Male responses after hearing both the control and the non-predator stimuli did not differ from before. Moreover, responses after hearing the non-predator stimulus compared to after hearing the control did not differ significantly (Fig. I-3; Table I-6). On the other hand, female responses to stimuli did not differ from the one-minute before or across stimuli (Fig. I-3; see Table I-6 for all comparisons and 95% CI's).

One-minute post-playback dewlap duration

We identified 11 candidate models that best explained the variation in dewlap duration after stimulus playbacks. The “best” model had a probability (w_i) of 0.17 and included the stimulus context (post -predator, -non-predator, and -control), PO, and the interaction between these two main effects. Context and PO were found in all models, while the interaction was found in seven models, WS in five models, PT in seven, and PH in six (Table I-3).

Thirty-seven lizards dewlapped before, during or after stimulus playbacks, however, one of these lizards was not included in post-predator treatment due to ‘escape’ during the predator playback. Summary statistics can be found in Table I-9. PT, PH, and WS did not have a significant effect on dewlap duration (Table I-4). On the other hand, we found a significant negative effect of the post-predator context and order (Fig. I-4; Table I-4). Male lizards significantly reduced their time dewlapping after hearing the predator and non-predator playbacks compared to the control when lizards heard these stimuli first (Table I-6). There was no difference in dewlap duration across stimulus contexts for the second and third positions (see Table I-6 for all comparisons and 95% CI's).

Discussion

Our results provide evidence that free-ranging male brown anoles use auditory cues to assess predation risk and that they were able to distinguish between threatening and non-threatening stimuli. Specifically, male anoles responded in a graded manner to the three stimuli as predicted by the threat-sensitivity hypothesis (Helfman 1989). Playback of the predatory kestrel call elicited the quickest response time and the greatest number of antipredator behaviors, indicating that males perceived this call as more threatening than the non-predatory cardinal call and white noise. Males also responded more quickly and were more vigilant during playback of the non-predator cardinal call relative to white noise. Furthermore, males remained more vigilant for the one-minute period following playback of the predatory kestrel call. Our findings are consistent with previous studies where lizards increased vigilance in response to playbacks of predatory bird vocalizations (Cantwell and Forrest 2013) and heterospecific alarm calls (Ito and Mori 2010; Vitousek et al. 2007).

The ability to recognize predatory vocalizations is more likely to occur when prey have been exposed to the predator within its lifetime and whose ancestors were also exposed to the predator (Hettena et al. 2014). The lizards from our population and the population tested in Cantwell and Forrest (2013) were from two different regions in Florida (St. Johns County and Brevard County, respectively) that overlap with the geographic range of resident and migratory kestrels (FWC n.d.). Furthermore, brown anoles are sympatric with American kestrels over much of their native geographic range in the West Indies and all of their introduced range in the southeastern United States. Given that, the small territory size of brown anoles and the sheer numbers of these anoles in a given area, the opportunity has existed for brown anoles to learn to associate kestrel calls with the risk of predation. The observed increase in vigilance seen in male anoles clearly shows

they recognize the call of the American kestrel and perceive it as threatening (the lizards in the current study; Cantwell and Forrest 2013). In contrast, Elmasri et al. (2012) found that brown anoles inhabiting an isolated island near Calabash Cay that was devoid of resident and migrating kestrels, did not respond when presented with auditory playbacks and/or a visual model of an American kestrel. Together, while these findings highlight the importance of learning for male anoles to discriminate predatory vocalizations, we cannot yet rule out the possibility of an innate response to auditory predator cues driven by strong and rapid selection pressure without further studies. Lizards, and reptiles in general, have typically been stereotyped as ‘cold-blooded’, ‘non-social’ animals that lack the ability to learn; however recent evidence contradicts this perception. For example, the water skink, *Eulamprus quoyii* (Scincidae) (Noble et al. 2014), the bearded dragon, *Pogona vitticeps* (Agamidae) (Kis et al. 2015), and the wall lizard, *Podarcis lilfordi* (Lacertidae) (Pérez-Cembranos and Pérez-Mellado 2015) are all capable of social learning. Moreover, at least some lizards exhibit taste aversion learning (Paradis and Cabanac 2004), a biologically relevant mechanism that has the potential to increase an individual’s survival.

Conversely, our findings provide mixed results for the use of auditory cues by free-ranging female brown anoles to assess predation risk. Female lizards, like males, responded more quickly to the predator vocalization, and to the bird calls in general, however, they exhibited similar levels of heightened vigilance before, during and after playback of all stimuli. Female responses to stimuli also contradict the general understanding that males are more responsive auditory cues (alarm calls) than females (Lea and Blumstein 2011). These findings were unexpected and present a more complex picture. Our playback experiments were conducted during the middle of the breeding season when approximately 90% of female lizards are gravid (Lee et al. 1989).

Female anoles allocate more energy to egg production during the breeding season (Orrell et al. 2004) and the associated reproductive load significantly decreases locomotor activity (Johnson et al. 2010) and performance (Shine 1980). Consequently, female lizards may shift to an increased state of wariness during the breeding season to offset the costs associated with reproduction. Heightened vigilance in females likely increases their chances of detecting a predator at which time they may adopt a ‘cryptic’ defense strategy to avoid predation (Schwarzkopf and Shine 1992). On the other hand, if learning is important for discrimination, then female lizards may be less likely to learn or slower to learn to discriminate between calls than males due to differences in their social role (Carazo et al. 2014). Additional studies are needed to determine the antipredator tactics used by female anoles outside of the breeding season and whether or not their responses to auditory cues are more selective.

Both male and female anoles were more likely to respond with antipredator behavior to the calls of the kestrel and the cardinal compared to white noise. Additionally, the kestrel call elicited a faster response to the white noise; however, response times to the calls of the two bird species did not differ significantly. Furthermore, male lizards decreased display duration following calls of both species of birds and only reduced display duration for white noise when it was played in the second or third position following one of the two bird calls. Combined, these findings suggest that these lizards were more responsive to the bird calls, in general, relative to white noise. There are four possible explanations for this. First, anoles may consider the cardinal as a threat, albeit a lesser threat than the kestrel. Northern cardinals typically feed on seeds, fruits, and insects, however, they have also been observed consuming brown anoles in Florida (Dufour 2015; Turnbough 2013). Second, call frequency may play a role in initial response to auditory stimuli. For example, studies show that peak frequency is an important characteristic of

call structure for the identification of both familiar and unfamiliar alarm calls (Fallow et al. 2013). Although the mean difference in peak frequency between the kestrel call (~4500 kHz) and the cardinal call (~3000 kHz) was ~1500 kHz, both frequencies fall within the range of greatest sensitivity for anole hearing (Brittan-Powell et al. 2010). Third, the sudden onset of the playbacks may have triggered a startle response (Koch 1999), although this is less likely given the average time it took lizards to respond. Finally, we found that wind speed had a significant effect on whether or not lizards responded with antipredator behavior, as well as on the time it took lizards to respond to the auditory stimuli. Lizards were less likely to respond and took longer to respond during playback of stimuli at higher wind speeds. If wind speed resulted in the playback being less loud, then lizards may not have perceived the call as being a great threat. We are unable to elaborate on the specific behavior exhibited in relation to perceived proximity because we did not measure playback intensity at lizard perch sites for the stimuli or determine the direction in which the wind was blowing. However, it is likely that lizards may alter their defense strategy depending on the perceived proximity of the threat (Nishiumi and Mori 2015).

Previous studies investigating behavioral responses to alarm calls suggest that males are typically more responsive than females; however, most studies have been biased towards males (Lea and Blumstein 2011). To our knowledge, our study is the first to compare the behavioral responses of male and female lizards to auditory cues and assess their ability to localize sounds in space. Male lizards were more likely to discriminate threatening and non-threatening stimuli, while female lizards were generally more alert even during baseline observations. Additionally, two-thirds of male lizards and half of female lizards looked in the direction of the speaker upon playback of stimuli; therefore, lizards are likely able to localize the source of auditory vocalizations, thereby providing important information about the risk of predation. Furthermore,

our results suggest that learning may play an important role in discrimination of stimuli by male lizards. A growing body of evidence suggests that lizards, like birds and mammals, use auditory cues to assess predation risk and that they are able to distinguish between threatening and non-threatening stimuli. Future studies are needed to understand the underlying mechanisms driving discrimination of auditory cues and determine the extent of the effects that seasonal variation may have on the antipredator strategies adopted by males and females.

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Appendix I

Table I-1. Ethogram of behaviors observed in male and female *Anolis sagrei*.

Category	Behavior	Description
Antipredator	Head movement	Any movement of the head in which it is turned along a horizontal plane to look to the left or right or at angle along a vertical plane in which it appears to be looking toward the sky, either to the left or right; lizard may also raise its head away from the branch or trunk or lower the head to be closer to the branch or trunk upon which it is perched
	Body shift	Any movement of the body in which a lizard takes approximately 1 - 3 steps to move to higher or lower location on the branch or trunk, or moves to the left or right; lizard remains in sight and in relatively same location
	Crouch	Flexion of front two legs or all four legs resulting in body and head being closer to branch or trunk on which it is perched
	Raise body	Extension of front two legs or all four legs resulting in body and head being raised away from branch or trunk on which it is perched
	Squirrel*	Abrupt lateral movement around perch during stimulus playback, effectively moving out of line-of-sight
	Escape*	Rapid movement away from location during stimulus playback; may move to an area of cover where it can't be seen
Locomotion	Site change*	Any movement of animal in habitat; lizard is no longer positioned at original place of sighting
Courtship/Territorial Displays	Dewlap*	Extension of gular flap produced by the erection of the retrobasal process of the hyoid apparatus upon the fulcrum of the basi-hyal component; often coordinated with head-bob and push-up
	Head-bob*	Vertical movements of the head; often coordinated with dewlap and push-up
	Push-up*	Raising and lowering of the forebody by rhythmic flexion and extension of the forelimbs; often coordinated with dewlap and head-bob

*modified from Greenberg 1977

Table I-2. Summary statistics of physical traits and environmental conditions for both male and female *Anolis sagrei* during testing.

Sex	Physical trait or Environmental condition	Mean $\pm$ SE	Range
Male			
	SVL (mm)	56.1 $\pm$ 0.5	49 – 62
	Weight (g)	5.88 $\pm$ 0.1	3.8 – 7.5
	Ambient temperature (C°)	31.1 $\pm$ 0.11	26.0 – 33.7
	Perch temperature (C°)	31.6 $\pm$ 0.1	26.8 – 35.6
	Perch height (m)	1.45 $\pm$ 0.3	0 – 3.96
	Wind speed (m/s)	1.04 $\pm$ 0.05	0 – 3.3
Female			
	SVL (mm)	42.2 $\pm$ 0.4	41 – 44
	Weight (g)	2.25 $\pm$ 0.1	1.8 – 2.7
	Ambient temperature (C°)	31.4 $\pm$ 0.2	26.6 – 34.6
	Perch temperature (C°)	31.9 $\pm$ 0.18	28 – 34.6
	Perch height (m)	0.86 $\pm$ 0.08	0 – 2.65
	Wind speed (m/s)	1.05 $\pm$ 0.07	0 – 2.5

Table I-3. Top ranked models within four ΔAICc of the "best" model for each analysis, where K = number of parameters, w_i = Akaike weight (probability that a model is the "best" model in the candidate set), and ER = evidence ratio (the odds ratio in favor of the "best" model over another). PO = playback order, WS = average wind speed (m/s), PH = perch height, and PT = perch temperature.

Analysis	Model	K	AICc	ΔAICc	w_i	ER
Antipredator Behavior	Stimulus + PO + WS	7	295.67	0	0.17	
	Stimulus + PO	6	296.3	0.63	0.13	1.37
	Stimulus + PO + WS + PH	8	296.82	1.15	0.1	1.78
	Stimulus + PO + WS + Sex	8	296.85	1.19	0.1	1.81
	Stimulus + PO + WS + ST	8	297.33	1.66	0.08	2.3
	Stimulus + PO + PH	7	297.41	1.75	0.07	2.39
	Stimulus + PO + Sex	7	297.48	1.81	0.07	2.47
	Stimulus + PO + PT	7	298.08	2.41	0.05	3.33
	Stimulus + PO + WS + PH + Sex	9	298.5	2.83	0.04	4.13
	Stimulus + PO + WS + PH + ST	9	298.56	2.89	0.04	4.24
	Stimulus + PO + WS + PT + Sex	9	298.64	2.97	0.04	4.42
	Stimulus + PO + PH + Sex	8	299.1	3.44	0.03	5.57
	Stimulus + PO + PH + PT	8	299.25	3.58	0.03	5.98
	Stimulus + PO + PT + Sex	8	299.35	3.69	0.03	6.31
	Stimulus + PO + WS + Sex + Stimulus:Sex	10	299.5	3.83	0.03	6.79
Latency to Respond	Stimulus + WS + PH	7	415.08	0	0.38	
	Stimulus + WS + PH + Sex	8	416.94	1.87	0.15	2.54
	Stimulus + PH	6	417.19	2.11	0.13	2.87
	Stimulus + WS + Sex	7	417.61	2.54	0.11	3.56
	Stimulus + WS	6	417.71	2.64	0.10	3.74
	Stimulus + WS + PH + PO	9	418.59	3.51	0.07	5.8
	Stimulus + PH + Sex	7	419.04	3.97	0.05	7.28
Vigilance - 20 sec	Context + Sex + Context:Sex + WS + PO	10	420.7	0	0.51	
	Context + Sex + Context:Sex + WS + PO + PT	11	422.35	1.64	0.22	2.27
	Context + Sex + Context:Sex + WS + PO + PH	11	422.71	2.01	0.19	2.73
	Context + Sex + Context:Sex + WS + PO + PT + PH	12	424.36	3.66	0.08	6.24
Vigilance - One minute before and after***QAICc	Context + Sex + Context:Sex + WS	10	635.83	0	0.58	
	Context + Sex + Context:Sex + WS + PT	11	637.88	2.04	0.21	2.73
	Context + Sex + Context:Sex + WS + PH	11	638	2.12	0.2	2.87
Display Duration - One minute post stimulus playback	Context + PO + Context:PO	11	358.96	0	0.17	
	Context + PO + Context:PO + WS	12	359.19	0.23	0.15	1.12
	Context + PO + Context:PO + PT	12	359.29	0.33	0.14	1.18
	Context + PO + PT + WS	9	360.13	1.16	0.09	1.79
	Context + PO + Context:PO + PT + WS + PH	14	360.21	1.24	0.09	1.86
	Context + PO + PT	8	360.45	1.48	0.08	2.10

Table I-3. Continued

Analysis	Model	K	AICc	Δ AICc	w_i	ER
	Context + PO + Context:PO + WS + PH	13	360.59	1.63	0.07	2.26
	Context + PO + Context:PO + PH	12	360.72	1.75	0.07	2.40
	Context + PO + PT + WS + PH	10	361.27	2.30	0.05	3.16
	Context + PO + Context:PO + PT + PH	13	361.37	2.41	0.05	3.33
	Context + PO + PT + PH	9	361.93	2.97	0.04	4.41

Table I-4. Model averaged parameter estimates from the candidate model set for each analysis.

Effects that are significantly different from 0 based on 95% CIs are shown in bold.

Analysis	Variable	Parameter estimate	SE	95% CI
Antipredator Behavior	Intercept	1.37	2.5	-3.53 – 6.26
	Perch temperature	0.08	0.13	-0.18 – 0.33
	Perch height	-0.31	0.35	-1.0 – 0.37
	Average wind speed	-0.47	0.28	-1.02 – 0.09
	Stimulus Type			
	Predator	1.62	0.47	0.71 – 2.53
	Non-predator	1.14	0.43	0.29 – 1.98
	Sex			
	Male	-0.47	0.55	-1.54 – 0.61
	Stimulus Type:Sex			
	Predator:Male	0.92	1	-1.04 – 2.89
	Non-Predator:Male	-0.52	1.08	-2.63 – 1.59
	Order			
	Second	-0.34	0.43	-1.19 – 0.5
	Third	-1.13	0.41	-1.94 – -0.32
Latency to respond	Intercept	2.04	0.27	1.5 – 2.57
	Perch height	-0.26	0.13	-0.51 – -0.01
	Average wind speed	0.22	0.11	0.01 – 0.43
	Stimulus Type			
	Predator	-0.59	0.18	-0.94 – -0.25
	Non-predator	-0.51	0.18	-0.86 – -0.17
	Sex			
	Male	-0.17	0.22	-0.59 – 0.26
	Order			
	Second	0.13	0.16	-0.18 – 0.45
	Third	0.02	0.17	-0.32 – 0.35
Vigilance - 20 second	Intercept	0.6	0.73	-0.82 – 2.02
	Perch temperature	0.02	0.03	-0.04 – 0.09
	Perch height	0.04	0.09	-0.14 – 0.23
	Average wind speed	-0.21	0.08	-0.37 – -0.06
	Context			
	Predator	0.24	0.19	-0.14 – 0.61
	Non-predator	0.2	0.2	-0.19 – 0.58
	Control	-0.29	0.22	-0.73 – 0.14
	Sex			
	Male	-0.71	0.21	-1.12 – -0.31
	Context:Sex			
	Predator:Male	0.78	0.23	0.33 – 1.23
	Non-Predator:Male	0.5	0.24	0.04 – 0.97
	Control:male	0.41	0.27	-0.11 – 0.94
	Order			
	Second	-0.29	0.1	-0.48 – -0.1
	Third	-0.45	0.1	-0.64 – -0.25
Vigilance - One minute before and after***QAICc	Intercept	1.94	0.473	1.01 – 2.79
	Perch temperature	-0.01	0.033	-0.06 – 0.04
	Perch height	0	0.088	-0.15 – 0.15
	Average wind speed	-0.23	0.066	-0.65 – -0.11
	Context			
	Post-predator	-0.22	0.143	-0.48 – 0.04
	Non-predator	0.04	0.132	-0.2 – 0.28
	Control	0.08	0.132	-0.17 – 0.32
	Sex			

Table I-4. Continued

Analysis	Variable	Parameter estimate	SE	95% CI
Display Duration - One minute post stimulus playback	Male	-0.6	0.153	-0.87 – -0.33
	Context:Sex			
	Post-Predator:Male	0.58	0.176	0.28 – 0.89
	Post-Non-predator:Male	0.11	0.165	-0.19 – 0.40
	Post-Control:Male	0	0.165	-0.3 – 0.30
	Intercept	5.49	4.53	-3.39 – 14.36
	Perch temperature	-0.22	0.13	-0.47 – 0.03
	Perch height	-0.34	0.33	-0.98 – 0.3
	Average wind speed	-0.47	0.26	-0.97 – 0.04
	Stimulus context			
	Post predator	-1.34	0.59	-2.5 – -0.18
	Post non-predator	-0.82	0.49	-1.77 – 0.13
	Order			
	Second	-1.19	0.56	-2.29 – -0.09
	Third	-1.26	0.50	-2.24 – -0.27
	Stimulus context:Order			
	Post predator:Second	1.81	0.62	0.59 – 3.03
	Post predator:Third	1.53	0.62	0.32 – 2.74
	Post non-predator:Second	1.24	0.63	0 – 2.47
	Post non-predator:Third	1.10	0.61	-0.10 – 2.30

Table I-5. Combined male and female latency to respond to playback stimuli. Treatment stimuli ($\chi^2 = 12.6$, DF = 2, $p = 0.002$), average wind speed ($\chi^2 = 4.18$, DF = 1, $p = 0.04$), and perch height ($\chi^2 = 5.15$, DF = 1, $p = 0.023$) had a significant effect on lizard response times.

Analysis	Stimulus	N	Mean $\pm$ SE	Range
Latency to Respond (sec)				
	Control	70	7.05 $\pm$ 0.726	0.10 – 19.5
	Non-predator	86	4.39 $\pm$ 0.552	0.13 – 18.9
	Predator	96	4.18 $\pm$ 0.504	0.04 – 19.2

Table I-6. Pairwise comparisons on the multi-level predictor variables that had significant 95% confidence intervals from the ‘best’ model in the candidate set for each analysis. Comparisons that are significantly different from one another, based on 95% CIs are shown in bold.

Analysis	Comparison	Estimate	SE	Z value	P value	95% CI
Antipredator Behavior	Predator - Non-predator	0.509	0.448	1.14	0.256	-0.370 – 1.39
	Predator – Control	1.64	0.435	3.77	<0.001	0.619 – 2.66
	Non-predator – Control	1.13	0.399	2.83	0.013	0.196 – 2.06
	Playback order					
	Second – First	-0.356	0.431	-0.826	0.687	-1.37 – 0.654
	Third – First	-1.13	0.413	-2.74	0.017	-2.10 – -0.165
Latency to Respond	Third – Second	-0.776	0.398	-1.95	0.125	-1.71 – 0.156
	Predator - Non-predator	-0.085	0.161	-0.529	0.857	-0.463 – 0.292
	Predator – Control	-0.588	0.175	-3.36	0.002	-0.997 – -0.178
	Non-predator – Control	-0.502	0.174	-2.88	0.011	-0.911 – -0.094
Vigilance - 20 second	Female					
	Before – Control	0.293	0.222	1.32	0.55	-0.277 – 0.862
	Before – Non-predator	-0.195	0.195	-0.999	0.750	-0.697 – 0.307
	Before – Predator	-0.235	0.191	-1.23	0.607	-0.726 – 0.256
	Control – Non-predator	-0.488	0.215	-2.27	0.105	-1.04 – 0.064
	Control – Predator	-0.528	0.212	-2.49	0.061	-1.07 – 0.0164
	Non-predator – Predator	-0.040	0.184	-0.218	0.981	-0.513 – 0.433
	Male					
	Before – Control	-0.119	0.149	-0.795	0.857	-0.504 – 0.266
	Before – Non-predator	-0.699	0.133	-5.25	<.0001	-1.04 – -0.357
	Before – Predator	-1.02	0.127	-8.05	<.0001	-1.35 – -0.695
	Control – Non-predator	-0.579	0.129	-4.47	<.0001	-0.912 – -0.247
	Control – Predator	-0.901	0.123	-7.33	<.0001	-1.22 – -0.585
	Non-predator – Predator	-0.322	0.102	-3.16	0.009	-0.584 – -0.060
	Playback order					
	Second - Third	0.157	0.104	1.509	0.131	-0.047 – 0.362
Vigilance – One-minute	Female					
	Before – post control	-0.0762	0.124	-0.614	0.9277	-0.395 – 0.243
	Before – post non-predator	-0.0402	0.124	-0.324	0.9883	-0.36 – 0.279
	Before – post predator	0.219	0.133	1.66	0.3462	-0.121 – 0.561
	Post control – post non-predator	0.0361	0.124	0.291	0.9914	-0.282 – 0.355
	Post control – post predator	0.296	0.133	2.22	0.1175	-0.0463 – 0.639
	Post non-predator – post predator	0.260	0.133	1.95	0.2072	-0.083 – 0.603
	Male					
	Before – post control	-0.0766	0.0879	-0.870	0.820	-0.302 – 0.149
	Before – post non-predator	-0.147	0.0867	-1.69	0.327	-0.37 – 0.076
	Before – post predator	-0.363	0.0824	-4.41	0.0001	-0.575 – -0.152
	Post control – post non-predator	-0.0705	0.0856	-0.823	0.843	-0.291 – 0.15
	Post control – post predator	-0.287	0.0812	-3.53	0.0024	-0.496 – -0.0779
	Post non-predator – post predator	-0.216	0.08	-2.70	0.0347	-0.422 – -0.0107
Display duration – One minute	First					
	Post control – Post non-predator	1.05	0.397	2.65	0.0218	0.123 – 1.99
	Post control – Post predator	1.63	0.397	4.10	0.0001	0.696 – 2.56
	Post non-predator – Post predator	0.571	0.423	1.35	0.367	-0.420 – 1.56
	Second					
	Post control – Post non-predator	-0.271	0.413	-0.656	0.789	-1.24 – 0.697
	Post control – Post predator	-0.166	0.418	-0.396	0.917	-1.14 – 0.815

Table I-6. Continued

Analysis	Comparison	Estimate	SE	Z value	P value	95% CI
	Post non-predator – Post predator	0.105	0.385	0.272	0.960	-0.799 – 1.01
	Third					
	Post control – Post non-predator	-0.033	0.407	-0.081	0.996	-0.987 – 0.921
	Post control – Post predator	0.092	0.405	0.227	0.972	-0.857 – 1.04
	Post non-predator – Post predator	0.125	0.406	0.308	0.949	-0.826 – 1.08

Table I-7. Number and proportion of lizards that did not respond or responded with antipredator behavior to playback stimuli. Treatment stimuli ($\chi^2 = 16.0$, DF = 2, $p = 0.0003$) and playback order ($\chi^2 = 8.22$, DF = 2, $p = 0.016$) had a significant effect on the likelihood of whether lizards responded with antipredator behavior.

Sex	Stimulus	No Response	Antipredator Response	Proportion Responded
Male	Control	28	53	0.654
	Non-predator	15	66	0.815
	Predator	9	75	0.893
Female	Control	6	17	0.739
	Non-predator	2	21	0.913
	Predator	3	21	0.875

Table I-8. Summary statistics for vigilance behavior for the 20 second period before and during playback stimuli and the one-minute period before and after hearing playback stimuli. Pairwise comparisons can be found in Table I-6 for both analyses.

Sex	Analysis	Context/Stimulus	N	Mean $\pm$ SE	Range	Sex	Analysis	Context/Stimulus	N	Mean $\pm$ SE	Range
Male						Female					
	Vigilance – 20 second	Before	84	1.02 $\pm$ 0.14	0 – 6		Vigilance – 20 second	Before	24	2.04 $\pm$ 0.25	1 – 5
		Control	80	1.16 $\pm$ 0.13	0 – 5			Control	23	1.52 $\pm$ 0.36	0 – 7
		Non-predator	80	2.08 $\pm$ 0.17	0 – 6			Non-predator	23	2.48 $\pm$ 0.42	0 – 7
		Predator	80	2.86 $\pm$ 0.25	0 – 10			Predator	24	2.58 $\pm$ 0.37	0 – 6
	Vigilance - One minute	Before	83	3.07 $\pm$ 0.27	0 – 10		Vigilance - One minute	Before	24	5.45 $\pm$ 0.55	1 – 10
		Post control	79	3.35 $\pm$ 0.31	0 – 11			Post control	22	5.95 $\pm$ 0.56	0 – 10
		Post non-predator	78	3.6 $\pm$ 0.26	0 – 10			Post non-predator	23	5.65 $\pm$ 0.71	0 – 13
		Post predator	79	4.47 $\pm$ 0.39	0 – 18			Post predator	23	4.39 $\pm$ 0.63	1 – 13

Table I-9. Duration (seconds) of anole dewlap displays for one minute before playbacks began and during each of the three one-minute periods following playback of the control, non-predator and predator stimuli. Stimulus ($\chi^2 = 8.85$, DF = 2, $p = 0.012$), control period ($\chi^2 = 12.9$, DF = 2, $p = 0.001$) and the stimulus by control period interaction ($\chi^2 = 10.6$, DF = 4, $p = 0.03$) had a significant effect on dewlap duration. See Table 1-6 for pairwise comparisons.

Control Period	Stimulus	N	Mean $\pm$ SE	Range
Before First	N/A	26	10.8 $\pm$ 1.75	0 – 43.8
	Post Control	15	7.4 $\pm$ 2.5	0 – 32.4
	Post Non-predator	11	3.3 $\pm$ 1.7	0 – 14.1
	Post Predator	11	0.39 $\pm$ 0.39	0 – 4.3
Second	Post Control	10	2.2 $\pm$ 1.5	0 – 14.2
	Post Non-predator	14	3.0 $\pm$ 1.8	0 – 19
	Post Predator	13	1.4 $\pm$ 1.4	0 – 17.7
Third	Post Control	12	1.1 $\pm$ 1.1	0 – 12.8
	Post Non-predator	12	1.7 $\pm$ 1.7	0 – 19.8
	Post Predator	12	1.7 $\pm$ 1.3	0 – 14.8

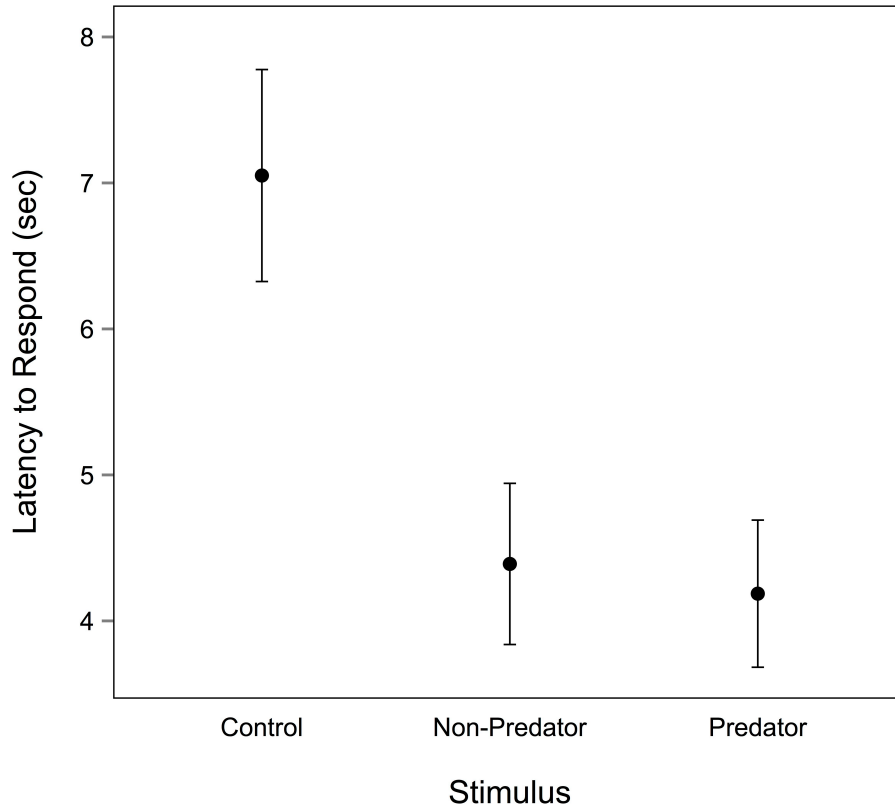


Figure I-1. Mean combined latency to respond ($\pm$ SE) for adult male and female *Anolis sagrei* to the control (N = 70), non-predator (N = 86), and predator (N = 96) stimuli. Lizards responded significantly faster to the predator ($p = 0.002$; 95% CI -0.997 – -0.178) and non-predator ($p = 0.011$; 95% CI -0.911 – -0.094) stimuli compared to the control. Response times to predator and non-predator stimuli were not significantly different ($p = 0.857$; 95% CI -0.463 – 0.292).

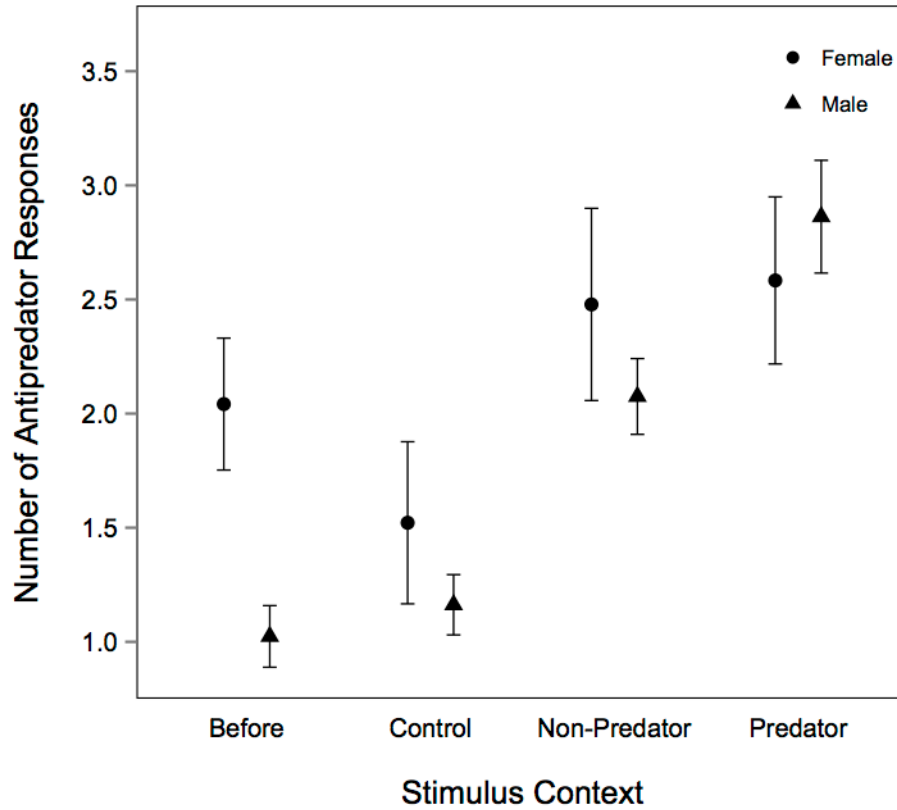


Figure I-2. Mean number of antipredator responses ($\pm$ SE) exhibited by female (solid circle) and male (solid triangle) adult brown anoles during the 20-second period before and during playbacks. Stimulus Context ($\chi^2 = 89.4$, DF = 3, $p = <2.2 \text{ e-}16$), the Stimulus Context by Sex interaction ($\chi^2 = 11.9$, DF = 3, $p = 0.008$), and wind speed ($\chi^2 = 7.02$, DF = 1, $p = 0.008$) had a significant effect on vigilance behavior. Sex had a marginally non-significant effect on vigilance behavior ($\chi^2 = 3.49$, DF = 1, $p = 0.06$). See Table I-6 for pairwise comparisons.

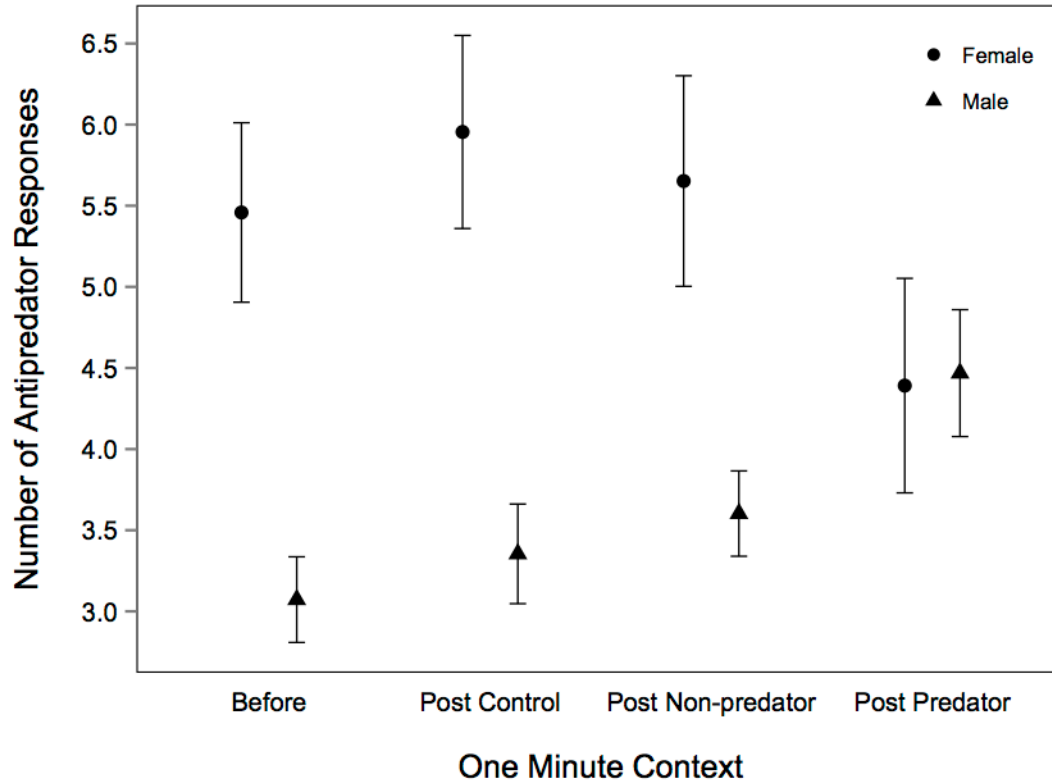


Figure I-3. Mean number of antipredator responses ($\pm$ SE) exhibited by female (solid circle) and male (solid triangle) adult brown anoles during the one-minute period before playbacks began and the one-minute period following playback of the control, non-predator, and predator stimuli. Stimulus Context ($\chi^2 = 9.97$, DF = 3, $p = 0.19$), Sex ($\chi^2 = 18.5$, DF = 1, $p = 1.7 \text{ e-}05$), the Stimulus Context by Sex interaction ($\chi^2 = 18.6$, DF = 3, $p = 0.0003$), and wind speed ($\chi^2 = 13.8$, DF = 1, $p = 0.0002$) had a significant effect on vigilance behavior. See Table I-6 for pairwise comparisons.

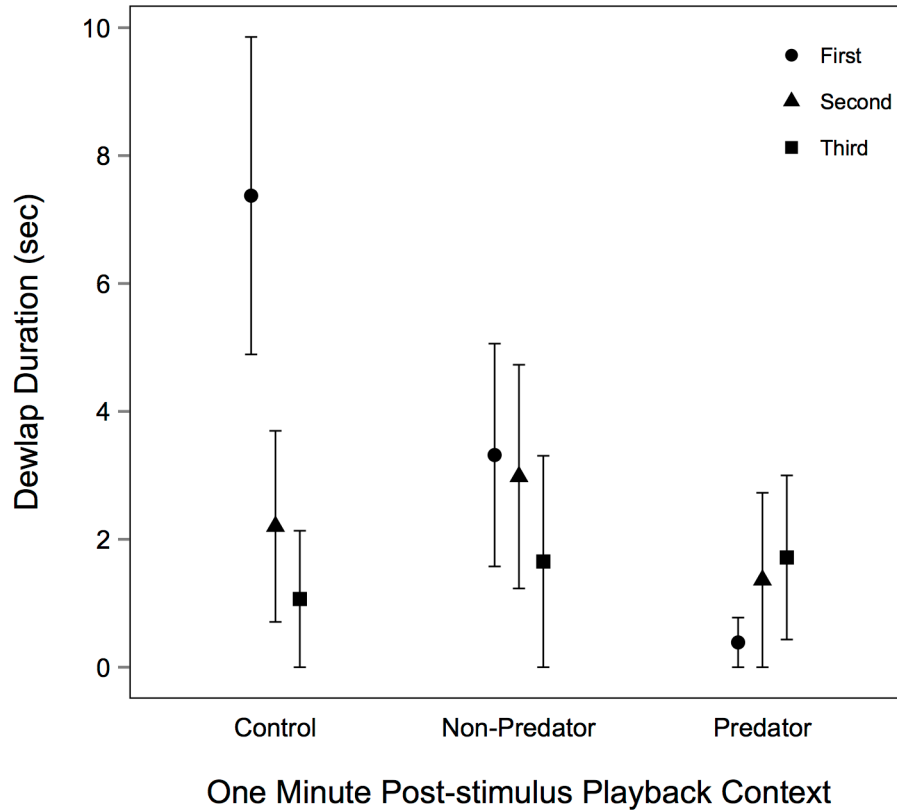


Figure I-4. Mean duration of dewlap displays ($\pm$ SE) exhibited by adult male brown anoles during the one-minute period following playback of the control, non-predator, and predator stimuli by the order of presentation. Stimulus Context ($\chi^2 = 8.85$, $DF = 2$, $p = 0.012$), the Control Period interaction ($\chi^2 = 12.9$, $DF = 2$, $p = 0.002$), and Stimulus Context by Control Period interaction ($\chi^2 = 10.6$, $DF = 4$, $p = 0.03$) had a significant effect on vigilance behavior. See Table I-6 for pairwise comparisons.

**CHAPTER II: THE USE OF AUDITORY DISTANCE CUES BY MALE BROWN
ANOLES TO ASSESS PROXIMITY OF AN AVIAN PREDATOR**

Abstract

Auditory cues are an important indicator of predation risk because they provide information about predator identity and proximity. Acoustic properties such as amplitude and sound pressure level can aid in distance perception thereby facilitating the location of a sound source. Prey that are able to utilize auditory distance cues to reduce detection by a predator are likely to increase their chances of survival. However, information about the use of auditory distance cues to assess predation risk is limited. We investigated male brown anoles' ability to acquire information about predator proximity from predatory kestrel vocalizations broadcast from three different distances, 4, 8, and 12 meters. Lizards responded faster to and were more vigilant during playbacks from 8 m compared to 4 and 12 m; however, there was no difference in time to respond or vigilance during playbacks between the 4 and 12 m treatments. In contrast, lizards were more vigilant after hearing playbacks from 4 m. Our study provides the first evidence that lizards, and likely prey animals in general, use auditory distance cues to assess predation risk and that lizards may utilize different antipredator strategies depending on risk level associated with predator proximity.

Introduction

The auditory system provides animals with information about their environment which enables them to find mates, navigate their surroundings, detect prey and even avoid predatory encounters (Yost 2007). Auditory cues can be detected earlier than visual and chemical cues (Weissburg et al. 2014) and at greater distances (Rosier and Langkilde 2011); therefore, they may help individuals locate a predator before a dangerous encounter, especially in dense vegetation or where visual detection of predators may be difficult. Furthermore, properties of acoustic cues, such as amplitude and changes in sound pressure level (SPL), can provide individuals with detailed information about the distance to the sound source thereby facilitating detection of the source location (Castellano et al. 2004; Naguib and Wiley 2001). For example, when distance from a sound source is doubled, sound energy decreases by 6 dB. Moreover, even small changes in SPL can be enough to yield a perceived change in distance (Naguib and Wiley 2001). Prey that can accurately perceive the distance of auditory predator cues and adjust behavior accordingly are likely to reduce the risk of predation and thus should be favored by selection.

Many studies have shown the importance of auditory distance cues in social contexts. For example, song birds use distance cues to estimate the location and proximity of territory intruders (Naguib 1996; Naguib et al. 2000; Phillmore et al. 1998) and frogs use distance cues to locate potential mates (Castellano et al. 2004; Murphy 2008). Specifically, studies on birds have shown the importance of SPL in accurately perceiving distance (Nelson 2000) and that amplitude was the most important property for assessing the distance of auditory cues (Radziwon et al. 2011). Additionally, Castellano et al. (2004) found that female green toads (*Bufo viridis*)

approach the louder of the same two calls when they are broadcast from the same distance but at different amplitudes.

In contrast, little is known about the use of auditory distance cues to assess predation risk. However, it is well known that animals recognize and respond to heterospecific alarm calls (primates: Gil-da-Costa et al. 2003; birds: Magrath et al. 2009; squirrels: Randler 2006; reptiles: Vitousek et al. 2007) and predator vocalizations (see review in Hettner et al. 2014). Consequently, it is likely that auditory distance cues can provide prey with information about the location and risk associated with predator proximity. We designed a simple study to investigate whether brown anoles (*Anolis sagrei*) use auditory distance cues (differences in playback amplitude) to assess predator proximity. Brown anoles increase vigilance in response to playbacks of predatory bird calls (Cantwell and Forrest 2013), therefore, we expected that calls played from closer distances would elicit stronger antipredator responses than calls played from further away.

Materials and Methods

Study species

Anolis sagrei is native to Cuba and the Bahamas, and was introduced into the southeastern United States in the 1940's (Bell 1953; Oliver 1950). Male *Anolis* are diurnal, social animals that use conspicuous displays during courtship and territorial defense (Greenberg 1977; Jenssen 1977), which may increase their risk of predation by avian predators (Costantini et al. 2007; Stuart-Fox et al. 2003; Vazquez and Hilje 2015). Although most anoles are non-vocal and typically thought of as being visually oriented (Losos 2009), they have well-developed auditory systems with enhanced directionality (Christensen-Dalsgaard and Manley 2005, 2008). Anoles have wide hearing sensitivity, ranging from 1 – 7 kHz (Brittan-Powell et al. 2010), which

overlaps the range of frequency (1 – 5 kHz) used by most birds to communicate (Dooling et al. 2000).

Audio clips and Playback

We obtained three different audio clips of the “killy” call of the American kestrel (*Falco sparverius*) from the Cornell Laboratory of Ornithology’s, Macaulay Library of Animal Sounds. This species was chosen because it is known to elicit increased vigilance in male brown anoles (Cantwell and Forrest 2013). Stimuli were 20 s in duration, normalized to 95% RMS peak amplitude using WavePad Sound Editor software v 4.59 (NCH Software Inc., Greenwood Village, CO, USA), and were broadcast through a Saul Mineroff SME-AFS field speaker (Elmont, NY, USA; 5 watts RMS; 100-12,000 Hz) positioned on an adjustable light stand, which was connected to a fourth generation iPod Nano (Apple Inc., Cupertino, CA, USA; model# 1285). The speaker was randomly placed either in front of the lizard or to the left or right side of the lizard; however, during some trials the lizard moved resulting in calls that were broadcast from behind the lizard. Speaker height was determined based on the perch height of the lizard such that a lizard ~ 1.3 m off the ground or less was tested at a speaker height of 1.52 m, a lizard between ~ 1.3 and 2.4 m off the ground was tested at a speaker height of 2.44 m and lizards that were higher than ~ 2.4 m were tested at a speaker height of 3.35 m.

Experimental Design

Playback experiments were performed on 63 adult male brown anoles during June and July of 2012 in St. Johns County, FL, USA (29° 53' N / 81° 18' W) between 0900 and 1500 hours when they are most active. We played kestrel calls at the same decibel level from three different distances: 4 (N = 21), 8 (N = 21) and 12 (N = 21) meters (± 0.1 m). The distance between the tree where the lizard was perched and the speaker was measured using a Bosch

Laser Distance Measurer (model# DLR130; ± 1.5 mm accuracy). When we visually located a lizard we placed the speaker at the designated distance (see above) and the digital camcorder (Sony Electronics Inc., San Diego, CA, USA; model# HDR-XR160) $\sim$ six meters from the focal subject. We stood motionless and allowed each lizard to acclimate to the observers' presence for three minutes prior to the start of testing after which we videotaped the lizard's behavior for two minutes before and after playback. To avoid lizards' habituating to the stimuli, lizards that were tested on the same day were separated by at least 50 m and experiments on different lizards but in similar localities to those already tested were conducted every third day. In order to prevent testing the same animal twice, we noosed lizards post-playback using a panfish telescopic fishing rod (Cabela's) and labeled them with a black sharpie; however, if we were unable to capture a lizard, we tied pink flagging tape around a branch on the tree and that tree was not tested again. After completion of each trial we recorded ambient temperature at 1 m from the ground (AT, $^{\circ}\text{C}$; Miller & Weber, Inc. cloacal thermometer), perch temperature at 1 cm from the perch (PT; $^{\circ}\text{C}$), minimum and maximum wind speed (WS, m/s; Kestrel 1000 Pocket Anemometer), canopy cover (CC, %; Forestry Suppliers, Spherical Crown Densiometer, Convex Model A), and perch height (PH, m; 2m Lufkin Red End Folding Rule) for each lizard tested, and snout-vent length (SVL; mm) and weight (W, g; 10g Pesola spring scale) for lizards that were captured. In addition, we measured the background decibel level of each testing location and took three readings of the perceived decibel level from the location of the lizard on the tree after each trial. Decibel level was measured using a Galaxy Audio Checkmate SPL meter (model# CM-140; time weighting: fast; frequency weighting: A; ± 1.5 dB).

Behavioral Observations

Videos were scored for three categories of behavior (courtship/territorial behavior, antipredator behavior, and locomotion; Table II-1). Lizard behavior was quantified for 1) two sequential one-minute periods prior to playback of the stimulus, 2) the 20-second playback stimulus, and 3) two sequential one-minute periods post playback. We recorded 1) the lizards' first response elicited by a stimulus (hereafter referred to as 'antipredator behavior'), 2) time to respond to the stimulus (hereafter referred to as 'latency to respond'), 3) whether the response was in the direction of the speaker, 4) total number of antipredator responses for each period of time (hereafter referred to as 'vigilance'), and 5) total duration and bout duration of courtship/territorial behaviors for each time period. To minimize observer bias, three observers, each blind to the treatment that the lizard received, scored videos using JWatcher v 1.0 video (Blumstein and Daniel 2007; <http://www.jwatcher.ucla.edu/>). Inter-observer reliability scores were calculated using Cohen's kappa (k); all scores were 'good' ($0.6 < k \leq 0.75$) or 'excellent' ($k > 0.75$) with a median 'excellent' score across all observers ($k = 0.886$; Kaufman and Rosenthal 2009).

Data Analysis

Although we conducted 63 total trials, we excluded any trials, or portion of a trial, during which we were unable to observe a lizard's behavior for longer than 20 seconds from the data analysis (see Results for sample sizes). We used an information-theoretic (IT) approach to model selection and multimodel inference (Burnham et al. 2011) to assess the effects of playback distance on anole behavior. All statistical analyses were conducted with R version 3.1.2 (R Core Team 2014).

We fit generalized linear models for antipredator behavior (binomial distribution), latency to respond (Gamma distribution, link = log), vigilance during playbacks (Poisson distribution), one-minute post vigilance (Poisson distribution), and two-minute post push-up/head bob duration (Gamma distribution, link = log). We used q-q plots to assess the fit of the residual error of each global model for normality. However, it was clear during exploratory analysis that the two-minute post playback dewlap duration was similar for all distances, therefore we used a one-tailed, paired Student's t-test to determine if there was a difference in dewlap duration before and after hearing playbacks, regardless of distance. In addition, we used a one-tailed, paired students t-test to determine if there was a difference in push-up/head bob durations before and after playbacks.

We conducted exploratory data analysis and did not find interactions between our independent variables; therefore, the global model for each analysis included distance, exemplar, perch height, perch temperature, and canopy cover as main effects. We did not include SVL or weight in the global models due to extremely small sample sizes for each treatment group ($N \leq 8$). Additionally, because brown anoles are ectothermic, environmental temperature could affect how or if a lizard responds to stimuli. We used perch temperature rather than ambient temperature because it should be a better predictor of the lizards' body temperature (Huey and Webster 1976).

We based model selection on Akaike's Information Criterion corrected for small sample size (AICc) except for when we detected overdispersion in the global model, in which case we estimated the variance inflation factor, $\hat{c}$, using the 'c_hat' function in the *AICcmodavg* package (Mazerolle 2015) and used quasi-AICc (QAICc) for model selection (Mazerolle 2006; Richards 2008). We fit all possible models for each analysis using the 'dredge' function in the package

MuMIn (Bartoń 2014). The traditional rule has been that models with $\Delta_i > 2$ are of little value for inference, however Anderson (2008) suggests that important information can be found in models with Δ_i between 2 and 4; therefore, we considered all models with $\Delta_i \leq 4$ relative to the best-fitting model as equivalent in explaining the variation in the response variable (Burnham et al. 2011). Next, we used various functions in the *AICcmodavg* package (Mazerolle 2015) to calculate the Akaike weights (w_i), evidence ratios (ER) and the model-averaged parameter estimates, unconditional standard errors, and unconditional 95% confidence intervals for all candidate models. We used the *multcomp* (Hothorn et al. 2008) and *lsmeans* (Lenth and Herv  2015) packages to conduct pairwise comparisons when multi-level predictor variables were significant. All pairwise comparisons were run from the ‘best’ model from the candidate set of models for all analyses. Confidence intervals were adjusted for multiple comparisons using the Tukey method and main effects were considered significant if they did not contain zero.

Results

General summary

Summary statistics for lizard physical traits and environmental conditions during testing can be found in Table II-2. Three lizards responded with a non-directional response (Crouch) to playbacks from Four m, four lizards responded in a direction different from where the speaker was placed, and eight lizards turned in the direction of the speaker. The speaker was directly behind the lizard for the remaining three lizards presented with playbacks from Four m. For the Eight m treatment, speaker direction was not recorded for two lizards; however, for the remaining lizards, 12 responded in the direction of the speaker, four responded in a different direction, two responded with non-directional responses (Raise head, Flight), and for one lizard the speaker was directly behind the lizard. Of the 13 lizards that responded to playbacks from

Twelve m, five responded in the direction of the speaker, two responded in a direction different from the speaker, and two responded with a non-directional response (Crouch, Flight). The speaker was directly in front of or behind the remaining four lizards.

Immediate effects

Latency to respond

Lizards that never responded to a stimulus were not included in the analysis. The ‘best’ model to explain variation in response times included Canopy cover and had a probability (w_i) of 0.49 (Table II-3). Canopy cover ($\chi^2 = 7.22$, $DF = 1$, $p = 0.007$) significantly affected lizard response times (Fig. II-2; Table II-4). Lizard response times were significantly faster under lower canopy cover compared to greater canopy cover (Table II-4). Summary statistics for latency to respond can be found in Table II-5.

Antipredator behavior

Antipredator behavior was observed in 85.7% lizards when presented with stimuli from Four m, 100% lizards from Eight m, and 65% lizards from Twelve m (Table II-7). The most common behavior observed in response to playbacks from all distances was head movement. Flight was observed in one lizard from Eight m and one lizard from Twelve m and the difference in playback intensity and background noise level was 27 dB and 22 dB, respectively.

The ‘best’ model to explain variation in whether or not lizards responded with antipredator behavior included Distance only and had a probability (w_i) of 0.33 (Table II-3). Even though there was a significant effect of Distance on the likelihood of lizards responding with antipredator behavior ($\chi^2 = 7.09$, $DF = 2$, $p = 0.029$; see Table II-4 for model averaged parameter estimates), we found no significant differences in antipredator behavior to playbacks

for pairwise comparisons of the three distances. However, there was a marginally non-significant difference between the Eight and Twelve m treatments (Table II-6).

Vigilance during playbacks

The ‘best’ model to explain the variation in vigilance during playbacks included Distance only and had a probability (w_i) of 0.35 (Table II-3). Playback distance had a significant effect on vigilance ($\chi^2 = 12.6$, DF = 2, $p = 0.002$; see Table II-4 for model averaged parameter estimates). Kestrel vocalizations played from Eight m elicited significantly greater vigilance in lizards compared to Twelve m while vocalizations played from Four m elicited a marginally non-significant heightened vigilance compared to Twelve m. There was no significant difference in vigilance to playbacks from Four and Eight m (Fig. II-3; Table II-6). Summary statistics for vigilance behavior during playbacks can be found in Table II-8.

Short-term effects

One-minute post-playback vigilance

One-minute vigilance behavior summary statistics can be found in Table II-8. The ‘best’ model to explain variation in vigilance during the one-minute period following playbacks included Distance and PT and had a probability of (w_i) 0.25 (Table II-3). Both Distance ($\chi^2 = 9.37$, DF = 2, $p = 0.009$) and PT ($\chi^2 = 5.78$, DF = 1, $p = 0.016$) had a significant effect on vigilance (see Table II-4 for model averaged parameter estimates). Lizards were significantly more vigilant for the one-minute period following playbacks of kestrel calls from Four m compared when heard from Twelve m. In addition, there was a marginal non-significant difference in vigilance following playbacks from Eight m compared to Twelve m. There was no difference in vigilance following playbacks from Four m and Eight m (Fig. II-4; Table II-6).

Lizards perched at higher temperatures elicited greater vigilance compared to lizards perched at lower temperatures (Table II-4).

Two-minute post-playback dewlap duration

On average, lizards dewlapped for similar durations following playbacks from all three distances (Table II-9; Fig. II-5a). However, lizards' dewlapped for significantly shorter durations after hearing playbacks compared to before hearing playbacks, regardless of the distance from which they were played ($t_{59} = -4.96$, $p < 0.001$; Table II-9; Fig. II-5b).

Two-minute post-playback push-up/head bob duration

In contrast, distance had a significant effect on push-up/head bob (PU/HB) duration ($\chi^2 = 12.7$, $DF = 2$, $p = 0.002$; Table II-4; Fig. II-5c). PU/HB duration was significantly shorter for lizards in the Four m treatment compared to the Twelve m treatment as well as the Eight m treatment compared to the Twelve m treatment (Fig. II-5c). However, there was no difference in duration between the Four m treatment and the Eight m treatment (Table II-6). In addition, PU/HB duration did not differ significantly before or after playbacks ($t_{59} = -1.18$, $p = 0.241$; Table II-9; Fig. II-5d).

Discussion

Our results provide some evidence that anoles may use distance as an important indicator of predation risk. Lizards were more vigilant during playbacks from Eight m compared to Twelve m suggesting that lizards perceive calls heard from a closer distance as posing a greater risk. However, we found no difference in the time it took lizards to respond to stimuli from the three distances. Moreover, there was no difference in the likelihood that anoles initially responded with antipredator behavior to predatory kestrel vocalizations regardless of playback distance. This may be due to the fact that the predator vocalizations were clearly audible from twelve

meters despite having a lower SPL (average SPL ~ 66 dB). Therefore, lizards are likely scanning the general area for a potential threat to acquire more information. Also, at all three distances, the lizards spent significantly less time dewlapping after hearing kestrel calls compared to before hearing calls. Vazquez and Hilje (2015) found that the presence of a conspicuous dewlap was more likely to make anoles more vulnerable to predation than when the dewlap was not extended or present. Furthermore, except with the special case of aposematic coloration, prey which have conspicuous body coloration have been found to be at greater risk of predation than those with less conspicuous coloration (Husak et al. 2006; Stuart-Fox et al. 2003). Consequently, the bright orange color of the dewlap may increase the likelihood that a potential [avian] predator would detect the lizard, even from a distance.

We found conflicting results for responses to playbacks from the Four m treatment compared to the Twelve m treatment. For example, lizards did not differ in vigilance during playbacks. While this was an unexpected result, lizards may adopt different antipredator strategies depending on the risk level associated with predator proximity. Nishiumi and Mori (2015) showed that frogs utilize different antipredator strategies depending on the distance between them and a snake predator; frogs were more likely to ‘flee’ when a snake was closest and ‘froze’ when a snake was more distant in an attempt to decrease detection. However, the lizards in our study rarely fled during or after hearing kestrel vocalizations, therefore, escape behavior may be dependent on having a visual stimulus present (see also Cantwell and Forrest 2013 and Simon 2007). On the other hand, anoles may ‘freeze’ when a predator is presumed to be nearby but is not in sight. However, only three lizards ‘froze’ (or ‘did not respond’) during playbacks from Four m and distinguishing between ‘freezing’ and ‘no response’ can be difficult given the absence of physiological evidence (e.g. changes in heart rate, blood pressure, and stress

hormones). On the contrary, lizards were more vigilant for the one-minute period following playbacks from Four m and also decreased the time spent producing push-up and head-bob displays for the two-minute period after playbacks from Four m compared to Twelve m. Similarly, Simon (2007) found that brown anoles reduce push-up displays following a simulated attack from an aerial predator. Anoles are cryptic in coloration therefore reducing movement would be particularly advantageous for brown anoles because it would decrease the probability of being detected. Furthermore, although we did not measure display amplitude, brown anoles have been found to reduce the amplitude of the head-bob display in areas with greater predation pressure (Steinberg et al. 2014).

Auditory cues can provide a wealth of information about predators if prey are capable of recognizing the cues and incorporating the information into decision making. To our knowledge, this is the first study to investigate the use of auditory distance cues as an indicator of predation risk. Our findings provide some evidence that anoles use auditory distance cues to assess predator proximity, thereby, likely reducing their risk of predation. Furthermore, we found evidence that anoles may utilize different antipredator strategies depending on risk level and that responses vary with time from stimulus exposure. The use of auditory distance cues to assess predation risk may therefore be more widespread throughout the animal kingdom. Future studies should consider measuring various physiological parameters in response to threatening auditory stimuli to be able to better assess whether prey are ‘freezing’ or ‘not responding’ to stimuli. Furthermore, studies investigating changes in physiological parameters in response to both threatening and non-threatening auditory stimuli will permit a greater understanding of the mechanisms driving antipredator behavior.

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Appendix II

Table II-1. Ethogram of behaviors observed in male brown anoles (*Anolis sagrei*) in response to playbacks of American kestrel (*Falco sparverius*) calls from 4, 8, and 12 m.

Category	Behavior	Description
Antipredator	Head movement	Any movement of the head in which it is turned along a horizontal plane to look to the left or right or at angle along a vertical plane in which it appears to be looking toward the sky, either to the left or right; lizard may also raise its head away from the branch or trunk or lower the head to be closer to the branch or trunk upon which it is perched
	Body shift	Any movement of the body in which a lizard takes approximately 1 - 3 steps to move to higher or lower location on the branch or trunk, or moves to the left or right; lizard remains in sight and in relatively same location
	Crouch	Flexion of front two legs or all four legs resulting in body and head being closer to branch or trunk on which it is perched
	Raise body	Extension of front two legs or all four legs resulting in body and head being raised away from branch or trunk on which it is perched
	Squirrel*	Abrupt lateral movement around perch during stimulus playback, effectively moving out of line-of-sight
	Escape*	Rapid movement away from location during stimulus playback; may move to an area of cover where it can't be seen
Locomotion	Site change*	Any movement of animal in habitat; lizard is no longer positioned at original place of sighting
Courtship/Territorial Displays	Dewlap*	Extension of gular flap produced by the erection of the retrobasal process of the hyoid apparatus upon the fulcrum of the basi-hyal component; often coordinated with head-bob and push-up
	Head-bob*	Vertical movements of the head; often coordinated with dewlap and push-up
	Push-up*	Raising and lowering of the forebody by rhythmic flexion and extension of the forelimbs; often coordinated with dewlap and head-bob

*modified from Greenberg 1977

Table II-2. Summary statistics of physical traits and environmental conditions for male *Anolis sagrei* during testing.

Physical trait or Environmental condition	N	Mean $\pm$ SE	Range
SVL (mm)	23	57.0 $\pm$ 0.49	52 – 63
Weight (g)	23	6.17 $\pm$ 0.15	5.2 – 7.4
Ambient temperature (C°)	63	29.9 $\pm$ 0.28	25.0 – 34.2
Perch temperature (C°)	63	30.1 $\pm$ 0.26	25.4 – 34.4
Perch height (m)	63	1.18 $\pm$ 0.07	0 – 3.02
Canopy cover (%)	63	82.2 $\pm$ 3.16	0 – 100
Background noise level (Hz)	63	49.2 $\pm$ 0.78	40 – 62.5

Table II-3. Candidate model set for each analysis within four ΔAICc of the “best” model. K = number of parameters, w_i = Akaike weight (probability that a model is the “best” model in the candidate set), ER = evidence ratio (the odds ratio in favor of the “best” model over another), LL = log likelihood, PT = perch temperature, PH = perch height, CC = canopy cover (%), PB Intensity = average playback intensity

Analysis	Model	K	AICc	ΔAICc	w_i	ER
Antipredator behavior	Distance	3	57.67	0	0.33	
	Distance + PT	4	59.00	1.34	0.17	1.95
	Distance + CC	4	59.61	1.94	0.13	2.64
	Distance + PH	4	59.90	2.23	0.11	3.06
	Distance + Exemplar	5	60.13	2.47	0.10	3.43
	Distance + PT + CC	5	60.92	3.25	0.07	5.08
	Distance + PT + PH	5	61.33	3.66	0.05	6.25
	PT	2	61.61	3.95	0.05	7.19
Latency to Respond	CC	5	264.82	0	0.49	
	PB Intensity + CC	3	266.14	1.32	0.25	1.93
	Null model	6	268.10	3.27	0.09	5.14
	CC + Exemplar	4	268.15	3.33	0.09	5.28
	PB Intensity	6	268.49	3.67	0.08	6.25
Vigilance - During**	Distance	4	178.10	0	0.35	
	Distance + PH	5	179.20	1.10	0.20	1.74
	Distance + PT	5	180.15	2.06	0.13	2.80
	Distance + CC	5	180.35	2.25	0.11	3.09
	Distance + Exemplar	6	181.27	3.17	0.07	4.89
	Distance + CC + PH	3	181.27	3.27	0.07	5.14
	Distance + PH + PT	6	181.53	3.43	0.06	5.57
Vigilance – One minute post**	Distance + PT	5	171.99	0	0.25	
	PT	3	173.09	1.10	0.15	1.73
	Distance	4	173.17	1.18	0.14	1.81
	Distance + PT + CC	6	173.79	1.80	0.10	2.45
	Distance + PT + PH	6	174.38	2.39	0.08	3.3
	PT + CC	4	175.20	3.21	0.05	4.97
	Distance + CC	5	175.20	3.21	0.05	4.98
	PT + PH	4	175.25	3.26	0.05	5.1
	CC	3	175.43	3.44	0.05	5.59
	PH	3	175.51	3.52	0.04	5.81
	Distance + PH	5	175.78	3.59	0.04	6.01

**QAICc used due to overdispersion

Table II-4. Model averaged parameter estimates from the candidate model set for each analysis.

Effects that are significantly different from 0, based on 95% CIs, are shown in bold.

Analysis	Variable	Estimate	SE	95% CI
Antipredator Behavior	Intercept	5.23	4.85	-4.28 – 14.74
	Perch temperature	-0.2	0.2	-0.59 – 0.2
	Perch height	-0.14	0.69	-1.49 – 1.2
	Canopy cover	-0.01	0.02	-0.06 – 0.03
	Distance			
	Four	-1.31	1.21	-3.69 – 1.06
	Twelve	-2.44	1.13	-4.67 – -0.22
	Exemplar			
	Playback 2	0.89	0.97	-1.02 – 2.8
	Playback 3	-0.46	0.82	-2.07 – 1.15
Latency to respond	Intercept	1.11	1.88	-2.58 – 4.79
	Average intensity	-0.03	0.03	-0.09 – 0.02
	Canopy cover	0.02	0.01	0.01 – 0.03
	Exemplar			
	Playback 2	0.31	0.30	-0.28 – 0.90
	Playback 3	0.43	0.31	-0.19 – 1.04
Vigilance - During	Intercept	0.88	0.79	-0.66 – 2.43
	Perch temperature	0.02	0.035	-0.08 – 0.13
	Perch height	0.19	0.17	-0.14 – 0.52
	Canopy cover	0	0	-0.01 – 0.01
	Distance			
	Four	-0.26	0.22	-0.69 – 0.17
	Twelve	-0.84	0.27	-1.37 – -0.32
	Exemplar			
	Playback 2	-0.1	0.23	-0.56 – 0.35
	Playback 3	-0.31	0.24	-0.78 – 0.17
Vigilance - One minute post	Intercept	-0.27	1.58	-3.36 – 2.81
	Perch temperature	0.08	0.04	-0.01 – 0.16
	Perch height	-0.04	0.16	-0.35 – 0.28
	Canopy cover	0	0	-0.01 – 0.01
	Distance			
	Four	0.15	0.2	-0.24 – 0.55
	Twelve	-0.34	0.22	-0.77 – 0.09

Table II-5. Mean latency to respond of male *Anolis sagrei* to stimuli from the three distances.

Canopy cover ($\chi^2 = 7.22$, DF = 1, $p = 0.007$) had a significant effect on lizard response times.

Analysis	Distance (m)	N	Mean $\pm$ SE	Range
Latency to Respond (sec)				
	Four	18	4.58 ± 0.948	0.12 – 13.1
	Eight	21	3.94 ± 1.01	0.14 – 16.9
	Twelve	13	5.93 ± 1.55	0.08 – 15.1

Table II-6. Pairwise comparisons of the three distances (4, 8, and 12 m) that had significant 95% confidence intervals from the ‘best’ model in the candidate set for each analysis. Distance effects that are significantly different from one another are shown in bold. P values and CI’s were adjusted for multiple comparisons using the Tukey method.

Analysis	Comparison	Estimate	SE	Z value	P value	95% CI
Antipredator Behavior	Eight - Four	1.25	1.20	1.05	0.542	-1.56 – 4.06
	Eight - Twelve	2.43	1.13	2.15	0.079	-0.213 – 5.06
	Four - Twelve	1.17	0.780	1.50	0.289	-0.656 – 3.00
Vigilance - During	Eight - Four	0.262	0.204	1.28	0.404	-0.216 – 0.741
	Eight - Twelve	0.845	0.251	3.37	0.002	0.257 – 1.43
	Four - Twelve	0.582	0.263	2.21	0.069	-0.034 – 1.120
Vigilance – One minute post	Eight - Four	-0.174	0.157	-1.11	0.5-9	-0.542 – 0.194
	Eight - Twelve	0.339	0.173	1.96	0.123	-0.067 – 0.745
	Four - Twelve	0.513	0.172	2.99	0.008	0.110 – 0.916
PU/HB duration – Two-minute post	Eight - Four	0.221	0.642	0.343	0.937	-1.28 – 1.72
	Eight - Twelve	-1.79	0.634	-2.83	0.013	-3.28 – -0.309
	Four - Twelve	-2.02	0.644	-3.13	0.005	-3.52 – -0.506

Table II-7. Number and proportion of lizards that did not respond or responded with antipredator behavior to playback stimuli. Distance ($\chi^2 = 7.09$, DF = 2, $p = 0.029$) had a significant effect on the likelihood of whether lizards responded with antipredator behavior.

Distance (m)	No Response	Antipredator Response	Proportion Responded
Four	3	18	0.857
Eight	0	21	1.0
Twelve	7	13	0.65

Table II-8. Summary statistics for vigilance for the 20 second period before and during playback stimuli and the one-minute period before and after hearing playback stimuli. See Table II-6 for pairwise comparisons.

Analysis	Distance	N	Mean $\pm$ SE	Range
Vigilance – 20 second	Four	21	2.24 ± 0.344	0 – 6
	Eight	21	2.91 ± 0.383	0 – 8
	Twelve	21	1.25 ± 0.270	0 – 4
Vigilance - One minute	Four	19	4.68 ± 0.536	1 – 9
	Eight	19	4.21 ± 0.651	0 – 11
	Twelve	19	3.00 ± 0.519	0 – 6

Table II-9. Duration (seconds) of anole displays for the two-minute period following playback of the stimuli from 4, 8, and 12 m. See Table II-6 for pairwise comparisons.

Display Type	Context/Distance	N	Mean $\pm$ SE	Range
Dewlap	Before	63	10.4 $\pm$ 1.42	0 – 43.1
	Four	19	3.16 $\pm$ 1.54	0 – 22.2
	Eight	19	3.80 $\pm$ 1.79	0 – 26.8
	Twelve	19	3.33 $\pm$ 1.50	0 – 23.7
	After	57	3.43 $\pm$ 0.916	0 – 26.8
Pushup/Head Bob	Before	63	3.54 $\pm$ 0.712	0 – 26.0
	Four	19	0.496 $\pm$ 0.281	0 – 5.0
	Eight	19	1.52 $\pm$ 0.862	0 – 12.2
	Twelve	19	5.07 $\pm$ 3.08	0 – 49.9
	After	57	2.36 $\pm$ 1.08	0 – 49.9

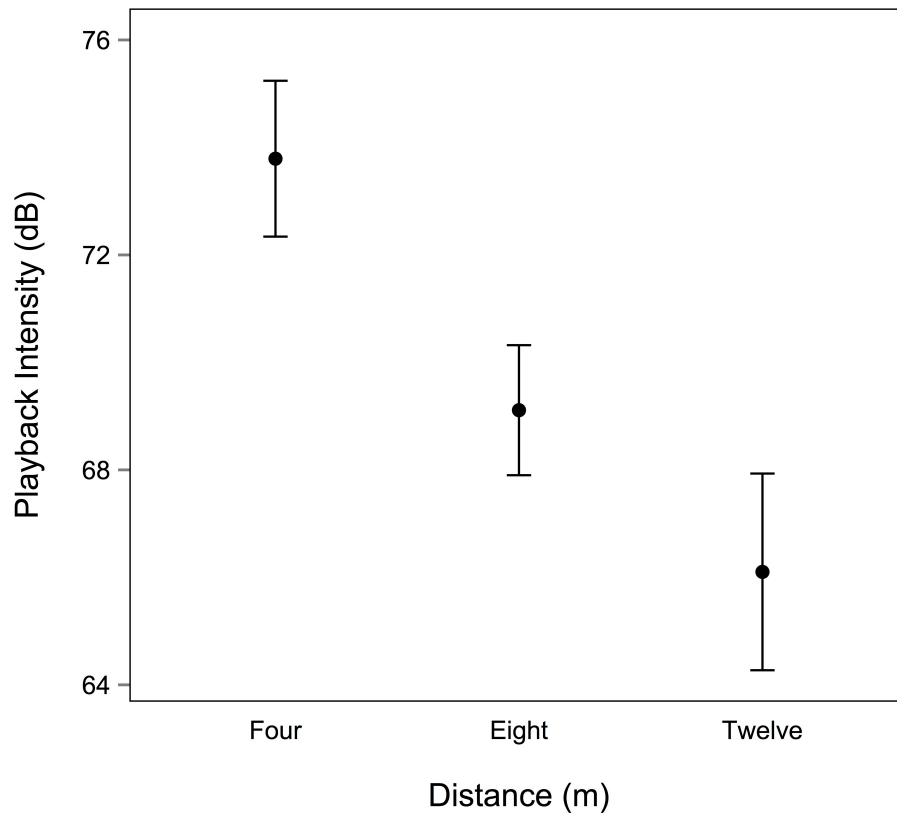


Figure II-1. Average playback intensity ($\pm$ SE) of kestrel calls from Four (73.8 ± 0.783 dB), Eight (69.1 ± 0.632), and Twelve (66.1 ± 0.493) meters. Distance had a significant effect on playback intensity ($\chi^2 = 57.8$, $DF = 2$, $p = 2.77 \text{ e-}13$).

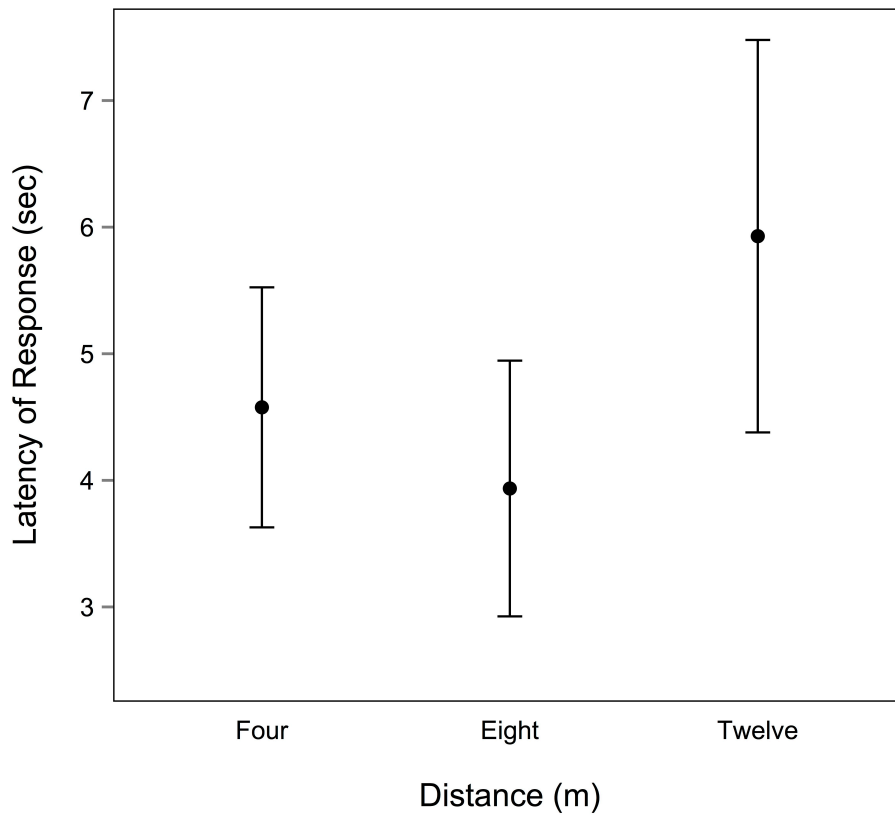


Figure II-2. Average latency to respond ($\pm$ SE) to kestrel calls by male *Anolis sagrei* from 4, 8, and 12 m. Canopy cover had a significant effect on response times ($\chi^2 = 7.22$, DF = 1, $p = 0.007$); lizards under greater canopy cover took significantly longer to respond to playbacks than lizards under less cover.

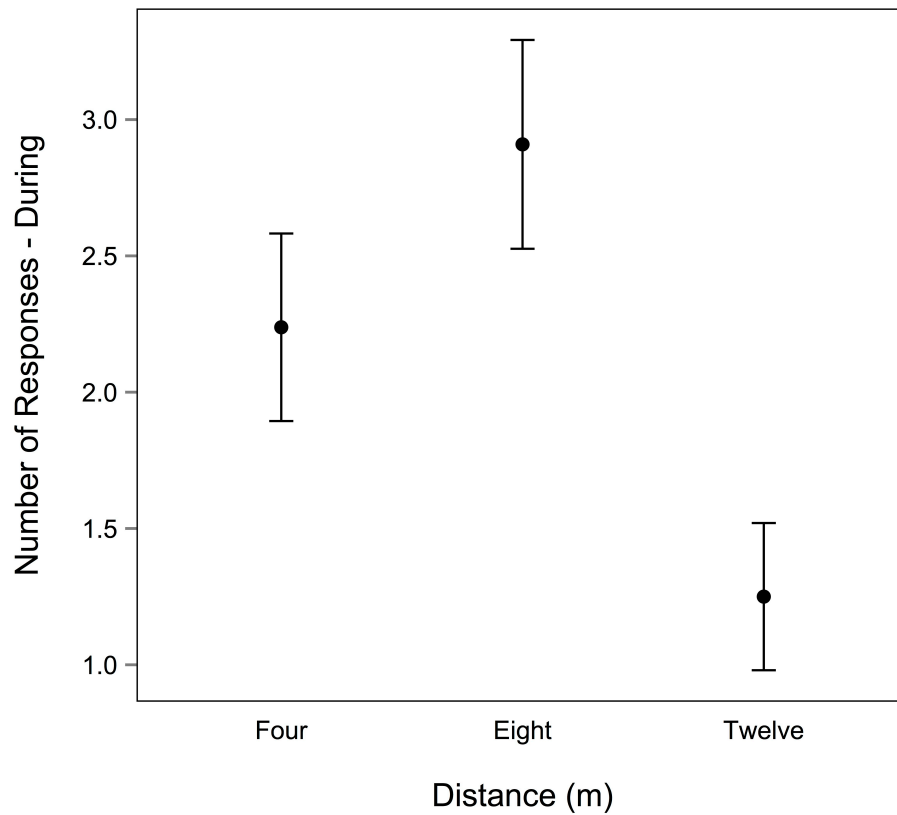


Figure II-3. The total number of antipredator responses ($\pm$ SE) elicited in lizards during playbacks of kestrel vocalizations from Four, Eight and Twelve meters. Lizards exhibited significantly greater vigilance to playbacks from Eight m compared to Twelve m (95% CI 0.257 – 1.43) while vocalizations played from Four m elicited a marginally non-significant heightened vigilance compared to Twelve m (95% CI -0.034 – 1.120). There was no significant difference in vigilance to playbacks from Four and Eight m (95% CI -0.216 – 0.741).

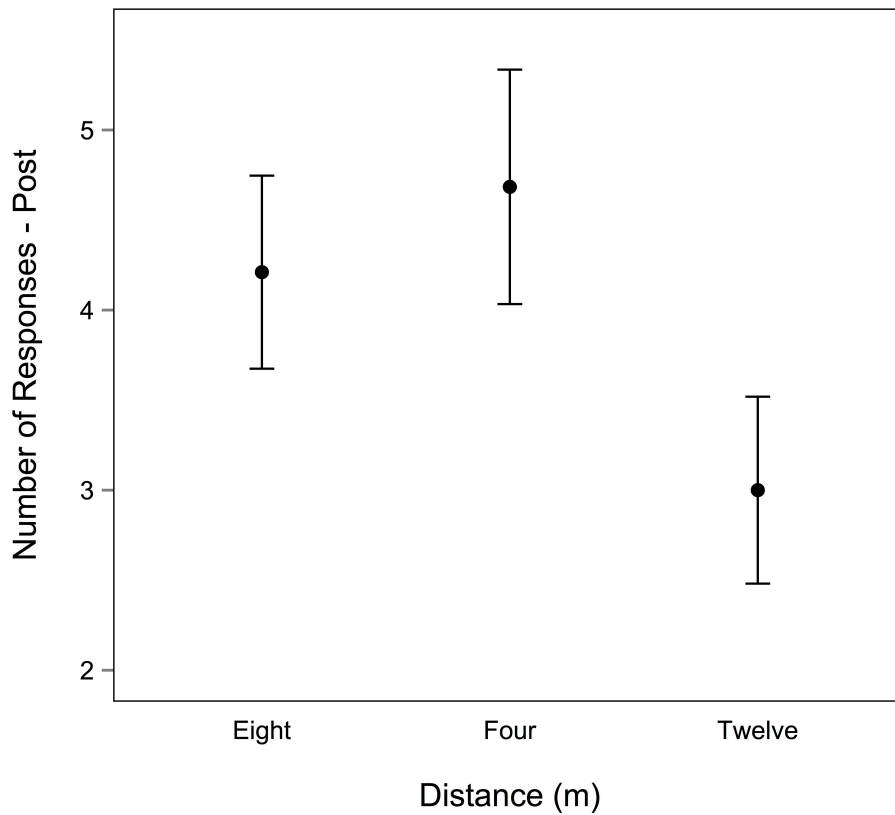


Figure II-4. The total number of antipredator responses ($\pm$ SE) elicited in anoles during the one-minute period following playback of kestrel vocalizations. Lizards were significantly more vigilant for the one-minute period following playbacks of kestrel calls from Four m compared when heard from Twelve m (95% CI 0.110 – 0.916). In addition, there was a marginal non-significant difference in vigilance following playbacks from Eight compared to Twelve m (95% CI -0.067 – 0.745). There was no difference in vigilance following playbacks from Four and Eight m (95% CI -0.542 – 0.194).

Figure II-5. Mean display durations ($\pm$ SE) during the two-minute period before and after playbacks of predator stimuli. (a) Dewlap duration for the two-minute period after hearing predator stimuli from 4, 8, and 12 m. (b) Dewlap duration for the two-minute period before and after hearing predator stimuli. Lizards dewlapped for significantly shorter durations after hearing playbacks compared to before hearing playbacks ($t_{59} = -4.96$, $p < 0.001$). (c) Push-up/head bob (PU/HB) duration for the two minute period following playbacks from 4, 8, and 12 m. Distance had a significant effect on PU/HB duration ($\chi^2 = 12.7$, $DF = 2$, $p = 0.002$). PU/HB duration was significantly shorter for lizards after hearing predator stimuli played from Four m compared to when played from Twelve m (95% CI -3.52 – -0.506) and the Eight m treatment compared to the Twelve m treatment (95% CI -3.28 – -0.309). There was no significant difference in PU/HB duration for the Eight m treatment compared to the Four m treatment (95% CI -0.790 – 2.90). (d) PU/HB duration for the two-minute period before and after hearing predator stimuli. PU/HB duration did not differ significantly after playbacks from before ($t_{59} = -1.18$, $p = 0.241$).

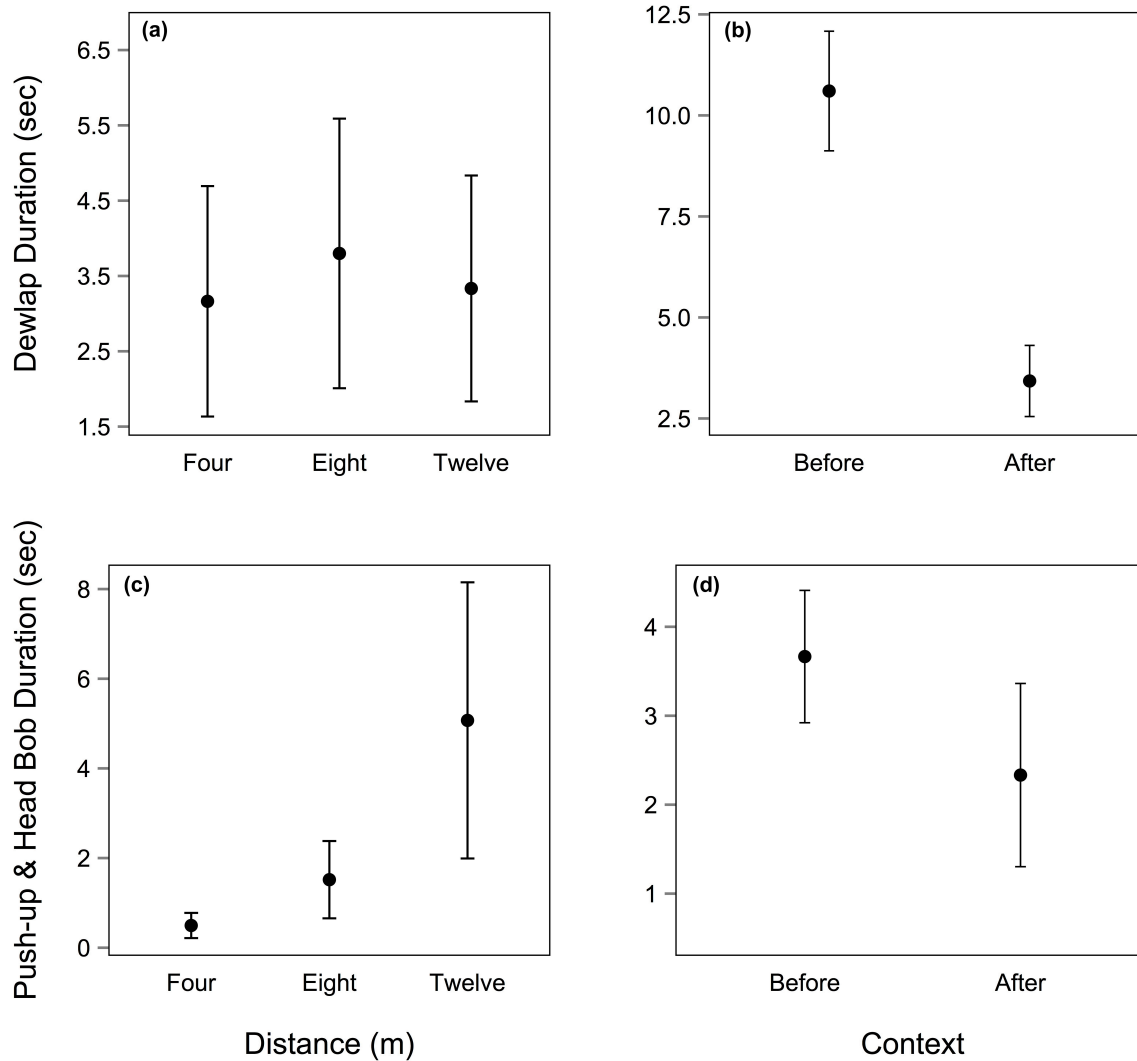


Figure II-5. Continued

**CHAPTER III: WHO'S THERE? THE IMPORTANCE OF FAMILIARITY IN
DISCRIMINATION OF AVIAN CALLS BY THE BROWN ANOLE**

Abstract

Numerous animals are able to recognize the vocalizations of their predators and incorporate the information gained about current predation risk into decision making. Most research to date investigating recognition of predator vocalizations has been conducted using calls with which prey have experience from birth and throughout their lifetime and are therefore familiar with calls; therefore, questions remain about the underlying mechanisms driving responses to predator vocalizations. To our knowledge, no studies have compared how prey respond to calls from a known predator, to predator vocalizations that exhibit similarity in acoustic properties, such as peak frequency, or similarity in call structure resulting from related, but allopatric species. This study sought to elucidate the mechanisms underlying antipredator responses in the brown anole (*Anolis sagrei*) by testing lizards with playbacks from a known sympatric avian predator, the American kestrel (*Falco sparverius*), and four increasingly distant but related, but allopatric saurophagous avian predators. We used an information theoretic approach to determine whether responses were best predicted by peak frequency, call similarity with regards to increasingly distant phylogenetic relatedness to the sympatric predator, or call familiarity (i.e. individual experience). We found that the model for peak frequency best predicted latency to respond and likelihood of responding with antipredator behavior. Also, the model for call familiarity best predicted vigilance both during playbacks and for the two-minute period following playbacks, in addition to time lizards remained alert for the two-minute period after playbacks. Our results suggest that recognition of predator vocalizations may be driven in combination by innate and learned mechanisms. We also found an anomaly in responses that may have resulted from testing lizards in semi-anechoic chambers and provide suggestions for future investigations.

Introduction

Animals that are unable to recognize a potential threat are at high risk of suffering severe fitness consequences (Lima and Dill 1990). Not unexpectedly, prey have evolved various mechanisms by which they are able to recognize potential predators. The general underpinnings of predator recognition involve the ability of prey to recognize threats from birth and/or prey learn to recognize predators through direct or indirect experience (Caro 2005). Furthermore, prey may utilize one or more sensory mechanisms to acquire information about predation risk, including auditory cues, such as conspecific (MacLean and Bonter 2013) or heterospecific (Magrath et al. 2015) alarm calls or predator vocalizations (Hettena et al. 2014). Prey that are able to recognize various auditory cues and incorporate information into decision making are likely to increase their chances of survival.

Predator vocalizations and alarm calls provide prey with important information about predation risk including (but not limited to): predator type, proximity or location, and threat level associated with the predator (Hettena et al. 2014; Magrath et al. 2015; Rosier and Langkilde 2011). A review by Hettena et al. (2014) found that the majority of predator vocalization playback studies were conducted on prey that had both evolutionary (i.e. the potential to possess an innate recognition) and ecological (i.e. within lifetime individual experience) experience with the predator and that these animals responded with heightened antipredator behavior. Similarly, MacLean and Bonter (2013) found that gulls were more likely to respond to vocalizations with which they were familiar and that responses also depended on the associated risk level.

Much research has been conducted on the ability of animals to recognize heterospecific alarm calls. Birds (Fallow et al. 2011; Fallow et al. 2013; Magrath and Bennett 2012), mammals

(Getschow et al. 2013; Kitchen et al. 2010) and even reptiles (Fuong et al. 2014; Ito et al. 2013; Ito and Mori 2010; Vitousek et al. 2007) have been shown to respond with antipredator behavior to playbacks of heterospecific alarm calls. However, the specific mechanisms underlying recognition of auditory vocalizations remain in question. For example, research on ungulates (Kitchen et al. 2010) and squirrels (Getschow et al. 2013) indicates that call familiarity is driving antipredator responses. In contrast, Fallow et al. (2011; 2013) investigated responses of Superb Fairy-wrens (*Malurus cyaneus*) to unfamiliar heterospecific alarm calls and found that peak frequency was an important factor driving antipredator responses.

To our knowledge, there is no study to date that has investigated whether recognition of predator vocalizations by prey is based on call familiarity or whether recognition is driven by specific call properties, such as peak frequency, or similarity in call structure resulting from closely related, but allopatric predators with which prey have no experience. Our study sought to add to our understanding of what drives prey recognition of predator vocalizations. We conducted a playback study on brown anoles (*Anolis sagrei*; Polychrotidae), which have been shown to recognize and respond to sympatric predatory American Kestrel (*Falco sparverius*) vocalizations (Cantwell and Forrest 2013; Cantwell and Echternacht unpublished data), with four different predatory vocalizations, a known sympatric predator, and three novel (allopatric) predators of increasing phylogenetic distance from the American Kestrel. We hypothesized that familiarity with predator vocalizations would be the driving factor of antipredator responses.

Materials and Methods

Study species

Anolis sagrei (Polychrotidae), the brown anole, is native to Cuba and the Bahamas and was introduced into the southeastern United States around the 1940s (Bell 1953; Oliver 1950).

We collected 32 adult male brown anoles during June 2012 in St. Johns County FL, USA (29° 53' N / 81° 18' W) between 0900 and 1500 hours EST. Lizards were transported to the University of Tennessee in Knoxville and housed individually in ventilated glass enclosures (3.79 l; 14 cm diameter). Each lizard was provided with crushed walnut shell substrate and a wooden perch. Lizards were maintained in a walk-in environmental chamber at an ambient temperature of $25 \pm 1^\circ\text{C}$, 50% relative humidity and a 12:12 hr light:dark cycle. All animals were misted daily and fed three crickets, dusted with RepCal calcium supplement, three times a week.

Predator Criteria and Auditory Stimuli

We used *F. sparverius* for the familiar (sympatric) predator because brown anoles increase vigilance in response to their calls (Cantwell and Forrest 2013) and their geographic range overlaps in both native (Cruz 1976) and introduced habitats (Giery 2013) where they are known to feed on anoles. Novel (allopatric) predators were chosen based on four criteria: 1) no shared evolutionary history with brown anoles, 2) increasingly distant phylogenetic relationship to the American kestrel, 3) similarity in size to the kestrel, and 4) lizards make up a significant part of their diet. The three novel predators used, in order of most closely related to most distantly related, were: 1) the Lesser Kestrel (*Falco naumanni*; Falconidae), 2) the White-rumped falcon (*Polihierax insignis*; Falconidae), and the Shikra (*Accipiter badius*; Accipitridae) (Hackett et al. 2008).

We obtained one audio clip of the *F. sparverius* from the Cornell Lab of Ornithology Macaulay Library of Animal Sounds and seven audio clips from Xeno-Canto (Fig. 1; <http://www.xeno-canto.org/>). We used two exemplars of each species call to reduce pseudoreplication (Kroodsma 1989). All audio clips were 20 seconds in duration and normalized

to 95% RMS peak amplitude with a 44.1 kHz sampling rate using WavePad Sound Editor software v 4.59 (NCH Software Inc., Greenwood Village, CO, USA). Auditory stimuli were broadcast through two Saul Mineroff SME-AFS (Elmont, NY, USA 5 watts RMS; 100-12,000 Hz) field speakers attached to a fourth generation iPod Nano (Apple Inc., Cupertino, CA, USA; model# 1285). Stimuli were broadcast at a sound pressure level (SPL) of 72.6 ± 1.5 dB (mean $\pm$ SD) measured at 1 m from the test enclosure using a Galaxy Audio sound meter (CM-140).

Experimental Design

We used a repeated measures design in which each lizard was exposed to one exemplar of each of the four treatments separated by 30 min of silence (e.g. stimulus 1 – silence – stimulus 2 – silence – stimulus 3 – silence – stimulus 4 – silence – stimulus 5). One exemplar from each of the treatments was randomly assigned to a playback order and each playback order was randomly assigned to a test day. Two lizards were tested simultaneously on the same day; therefore, each pair of lizards received the same playback order. Experimental trials were conducted during December 2012 and January 2013 between 0900 and 1500 hrs. Seven days prior to testing, two lizards were moved from their maintenance enclosures to separate screen test enclosures, identical in setup and size to their maintenance enclosure. The seven-day acclimation period was chosen to reduce stress associated with a new enclosure (Langkilde and Shine 2006). Lizards were maintained in the environmental chamber during the seven-day acclimation period. We used screen enclosures during testing to minimize reverberations from sound stimuli.

We moved two lizards from the environmental chamber to two separate semi-anechoic sound chambers (Med Associates Inc., St. Albans VT, USA) 24 hrs prior to testing for acclimation. Sound chambers were maintained at $23.7 \pm 1.1^\circ\text{C}$ (mean $\pm$ SD), 28.8 ± 2.8 %

(mean $\pm$ SD) relative humidity, and 12:12 hr light:dark cycle. A large blind with holes just large enough for the lens of the video cameras was hung in front of each sound chamber to block the subjects' view of the observer and playback equipment. We recorded the lizard's behavior with a Sony digital video camera (Sony Electronics Inc., San Diego, CA, USA; model# HDR-XR160). To reduce habituation to the auditory stimuli, the sound chambers were kept in a separate location from the environmental chamber. We recorded snout-vent length (SVL), tail length (TL) and weight (WT) for all lizards after testing. Each lizard was tested one time.

Behavioral Observations

Videos were scored for four categories of behavior (courtship/territorial behavior, locomotion, antipredator behavior, and eye state; Table 1). Behavior of each lizard was quantified for 1) two minutes prior to stimulus playback, 2) the 20-second period during playback, and 3) two minutes post stimulus playback. For each lizard, we recorded 1) the time it took to respond to the stimulus (hereafter referred to as 'latency to respond'), 2) the first response elicited by the stimulus (hereafter referred to as 'antipredator behavior'), 3) the total number of antipredator responses during playbacks (hereafter referred to as 'Vigilance'), 4) the total number of antipredator responses for the two-minute period post-playback, and 5) the time a lizard remained alert (see Table 1) for the two minute period following playback (hereafter referred to as 'alertness'). Four observers, three of whom were blind to treatment, scored videos of the behaviors using JWatcher v 1.0 video (Blumstein and Daniel 2007; <http://www.jwatcher.ucla.edu/>). Inter-observer reliability scores were calculated using Cohen's kappa (k); all scores were 'good' ($0.6 < k \leq 0.75$) or 'excellent' ($k > 0.75$) with an median 'excellent' score across all observers ($k = 0.89$; Kaufman and Rosenthal 2009).

Data Analysis

Model selection for acoustic similarity

Peak frequency was measured for all notes for each of the eight sound files using Avisoft SASLab Pro (Avisoft Bioacoustics, Glienicke, Germany). We then determined four different groups in which calls were classified as either low, medium, medium-high, or high frequency based on mean peak frequency for the call (Table III-2; Fig. III-2). We then fit four linear models for which each frequency classification group was used as the explanatory variable and peak frequency of the notes was the dependent variable. We used model selection and Akaike's Information Criterion corrected (AICc) for small sample size to determine which classification group best explained the variation in call frequency (Burnham et al. 2011; Table III-3). The top model was used in the final data analysis (see below).

Model selection for responses to playbacks of calls

We conducted a total of 160 trials (32 lizards x 5 stimuli) but excluded 18 trials from the data analysis in which the behavior of a lizard was unable to be observed (see Results for sample sizes). We used an information-theoretic (IT) approach to model selection (Burnham et al. 2011) to determine which model (familiarity with call, acoustic similarity between calls, or phylogenetic relation between avian species) best explained the variation in anole behavior. Because we did not detect overdispersion in any of the models (Mazerolle 2006; Richards 2008), model selection was based on Akaike's Information Criterion corrected (AICc) for small sample size (penalty based on the total number of both fixed and random effects) (Mazerolle 2006). For all tests, we fit generalized linear mixed model's (GLMM) with a random intercept for each individual, to account for non-independence in responses due to testing each lizard with multiple stimuli. Additionally, model parameters were estimated using adaptive Gauss-Hermite

quadrature approximation (Bolker et al. 2009). We calculated Akaike weights (w_i) and evidence ratios (ER) for all models using the *AICcmodavg* package (Mazerolle 2015). Next, we used the *multcomp* (Hothorn et al. 2008) and *lsmeans* (Lenth and Herv  2015) packages to conduct pairwise comparisons when multi-level predictor variables were significant. All pairwise comparisons were run from the ‘best’ model for all analyses. Confidence intervals were adjusted for multiple comparisons using the Tukey method and effects were considered significant if they did not contain zero. All statistical analyses were conducted with R version 3.1.2 (R Core Team 2014).

Results

Immediate effects

Latency to respond

Lizards that never responded to a stimulus were not included in the analysis. The best model to explain the variation in response times was the acoustic similarity model based on frequency classification and had a probability (w_i) of 0.26 (Table III-4). Frequency classification had a marginally non-significant effect on response times ($\chi^2 = 8.14$, $DF = 4$, $p = 0.087$; Table III-5). Although anoles responded most quickly to the Control stimulus and slowest to stimuli classified as High frequency, we found no significant differences in response times (Fig. III-3).

Antipredator behavior

Summary statistics for antipredator behavior can be found in Table III-6. The acoustic similarity model based on frequency classification, which had a probability (w_i) of 0.76, was found to be the best model to explain the variation in antipredator behavior (Table III-4). However, frequency did not have a significant effect on the likelihood on whether or not anoles exhibited antipredator in response to stimuli, as anoles were equally likely to respond with

antipredator behavior regardless of the frequency classification ($\chi^2 = 4.54$, $df = 4$, $p = 0.34$; Table III-7).

Vigilance – During playbacks

The familiarity model, based on whether the birds were sympatric or allopatric with brown anoles, was found to best explain the variation observed in vigilance during playbacks ($w_i = 0.46$; Table III-4). Although we did not find a significant effect of ‘patry’ on vigilance ($\chi^2 = 2.81$, $df = 2$, $p = 0.246$; Table III-7), playbacks of the sympatric predator calls elicited more antipredator behaviors in lizards than allopatric predator calls and the control (Fig. III-4).

Summary statistics for vigilance can be found in Table III-8.

Short-term effects

Vigilance – Two minutes post playback

Summary statistics for two-minute post vigilance can be found in Table III-8. Vigilance during the two-minute period post-playback was best explained by the model for familiarity with the call ($w_i = 0.56$; Table III-4). ‘Patry’ had a significant effect on post-playback vigilance ($\chi^2 = 32.5$, $df = 2$, $p = 8.7e-08$; Table III-7). Anoles were significantly more vigilant after hearing playbacks of the sympatric predator calls compared to allopatric predator calls. In contrast, anoles were significantly less vigilant after hearing allopatric calls compared to the control. There was marginally non-significant difference in vigilance between sympatric calls and the control (Table III-9; Fig. III-5).

Alertness

The model best explaining the variation in ‘alertness’ to calls post-playback was the model for familiarity with the call ($w_i = 0.81$; Table III-4). Patry had a significant effect on ‘alertness’ ($\chi^2 = 8.63$, $df = 2$, $p = 0.013$; Table III-7). Anoles were significantly more alert after

hearing the sympatric predator call compared to the allopatric predator calls and the control.

In comparison, there was no difference in lizard ‘alertness’ after hearing the allopatric calls compared to the control (Table III-9; Fig. III-6). Summary statistics for ‘alertness’ can be found in Table III-10.

Discussion

Our study sought to elucidate the mechanisms underlying anole responses to sympatric and allopatric predator vocalizations. We found that the acoustic similarity model, based on mean call frequency, best predicted the likelihood of anoles responding with antipredator behavior as well as latency of response to playbacks. In contrast, we found that the call familiarity model best predicted vigilance both during and after hearing calls in addition to the time lizards remained alert for the two-minute period following playbacks. Combined, these models suggest that antipredator responses to predator vocalizations are driven by both innate and learned mechanisms.

Call frequency best predicted antipredator responses and time to respond to playbacks. Lizards responded quickest to calls in the medium-high frequency classification (1.8s) which included both *F. sparverius* exemplars (mean frequencies 4407, 4512 Hz) and one *A. badius* exemplar (mean frequency 4380 Hz). These results are similar to those of Fallow et al. (2011; 2013) who found that peak frequency was an essential property affecting Superb Fairy-wren (*Malurus cyaneus*) responses to heterospecific alarm calls and synthetic calls, respectively. Our findings imply that call frequency is important in triggering an antipredator response and that the range of hearing frequency in brown anoles may have evolved as an ‘alerting’ mechanism to a potential threat. However, lizards responded to the white noise stimulus with the second fastest time at ~ 2.5 seconds which is more than three times faster than brown anoles in the wild

responded to the same white noise stimulus (Cantwell and Echternacht unpublished data).

Furthermore, unexpectedly, almost all lizards responded with antipredator behavior to all stimuli, including white noise, regardless of mean call frequency which also contradicts previous findings (Cantwell and Forrest 2013). We believe these to be the result of a possible artifact from testing the lizards in the semi-anechoic sound chambers resulting in an ‘unnatural’ silence throughout testing. It is possible that animals perceive silence as a signal that ‘danger is near’ and consequently stressful (J. B. Tennessen, personal communication, July 1, 2013). To our knowledge, this is the first report of semi-anechoic chambers potentially affecting responses to playback stimuli. Future studies involving the use of anechoic chambers should consider making comparisons to experiments conducted under more natural conditions. In addition, research should be conducted to determine if anechoic chambers do produce a physiological change such as an increase in stress hormones.

Call familiarity best predicted vigilance during and after playback of vocalizations accompanied by the time lizards remained alert after playbacks. Despite the fact that we did not find a statistical difference in vigilance during playbacks, lizards exhibited almost two times the number of head tilts during playbacks and remained alert more than twice as long after hearing the sympatric kestrel call. This suggests that recognition of vocalizations requires learning through individual experience. Previous studies on brown anoles also indicate that learning and predator experience is important in responding with antipredator behavior to predator vocalizations. For example, a population of brown anoles from Calabash Caye, Belize, that are not under risk of predation by kestrels do not respond with antipredator behavior to their vocalizations or a visual stimulus (Elmasri et al. 2012) whereas anoles living sympatrically with kestrels increase vigilance and decrease territorial/courtship signals upon hearing kestrel

vocalizations (Cantwell and Echternacht unpublished data; Cantwell and Forrest 2013; Huang et al. 2011) or when presented with a visual model (Simon 2007). Moreover, the Calabash Caye anole population, when presented with a playback and/or a visual model of a sympatric predatory grackle, did exhibit antipredator behavior. Therefore, it is possible that the population of anoles from Calabash Caye may have lost the antipredator response to the kestrel due to ‘adaptive forgetting’ related to increased uncertainty in predation risk (Ferrari et al. 2010) or the effects of being isolated on a small island (Blumstein and Daniel 2005).

Call frequency and familiarity were found to be more important in recognizing avian predator vocalizations than phylogeny. Recent research has suggested that more closely related birds have more similar calls and that receivers were more likely to respond to closely related species (Sosa-Lopez et al. 2016). Nevertheless, the lizards in our study did not respond more often with antipredator behavior or with an intensity that might be expected when hearing calls of more closely related species to *F. sparverius*. This may be due to the fact that these lizards are non-vocal, therefore, structural similarities between bird calls may not be recognizable. Moreover, the overlapping frequency sensitivity of anole ears to bird calls, in general, suggests that it could have evolved over time as a reliable indicator of danger, especially considering the historical aspect of living in shared environments with many [predatory] bird species. Importantly, if the frequency range of hearing evolved as an ‘alerting’ mechanism to a potential threat, then lizards would have an increased opportunity to learn to associate various calls with differing levels of threat.

This study provides further support that anoles are able to discriminate between auditory cues and recognize the vocalizations of sympatric avian predators as threatening. Critically, it also provides insight into the underlying mechanisms that prey use to recognize predatory

vocalizations. Prey that are able to gain information from heterospecific signals, in general, and respond accordingly are more likely to reduce their risk of predation (Caro 2005; Ferrari et al. 2012; Lima and Dill 1990).

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Appendix III

Table III-1. Ethogram of behaviors observed in captive male brown anoles (*Anolis sagrei*) in response to playbacks of sympatric and allopatric predatory bird calls.

Category	Behavior	Description
Antipredator	Head movement	Any movement of the head in which it is turned along a horizontal plane to look to the left or right or at angle along a vertical plane in which it appears to be looking toward the sky, either to the left or right; lizard may also raise its head away from the branch or trunk or lower the head to be closer to the branch or trunk upon which it is perched
	Body shift	Any movement of the body in which a lizard takes approximately 1 - 3 steps to move to higher or lower location on the branch or trunk, or moves to the left or right; lizard remains in sight and in relatively same location
	Crouch	Flexion of front two legs or all four legs resulting in body and head being closer to branch or trunk on which it is perched
	Raise body	Extension of front two legs or all four legs resulting in body and head being raised away from branch or trunk on which it is perched
	Squirrel*	Abrupt lateral movement around perch during stimulus playback, effectively moving out of line-of-sight
	Escape*	Rapid movement away from location during stimulus playback; may move to an area of cover where it can't be seen
Eye State	Alert (state)	Eyes open and observant of surroundings
	Relaxed (state)	Eyes closed and unobservant; usually resting

*modified from Greenberg 1977

Table III-2. Mean peak frequency, frequency range, and various frequency classifications for the eight avian exemplars used during testing.

Species exemplar	# of Notes	Mean Peak Frequency $\pm$ SD (Hz)	Frequency Range	Class One	Class Two	Class Three	Class Four
<i>F. sparverius 1</i>	94	4407 $\pm$ 316	3830 - 5210	Medium-high	High	Medium	High
<i>F. sparverius 2</i>	75	4512 $\pm$ 246	3960 - 4860	Medium-high	High	Medium	High
<i>F. naumanni 1</i>	12	4885 $\pm$ 397	4340 - 5550	High	High	High	High
<i>F. naumanni 2</i>	9	4107 $\pm$ 195	3870 - 4390	Medium	High	Medium	High
<i>P. insignus 1</i>	53	1370 $\pm$ 435	730 - 3180	Low	Low	Low	Low
<i>P. insignus 2</i>	40	1928 $\pm$ 765	990 - 3230	Low	Low	Low	Low
<i>A. badius 1</i>	21	4380 $\pm$ 491	3740 - 5380	Medium-high	High	Medium	High
<i>A. badius 2</i>	20	3600 $\pm$ 448	2450 - 4040	Medium	Medium	Medium	High

Table III-3. The four classification models used to determine the best call frequency classification to be used in the final data analysis. Models are ranked from best (top – lowest AICc) to worst (bottom – highest AICc) for each classification. See Table 2 for information pertaining to exemplar frequencies and classifications. K = number of parameters, w_i = Akaike weight (probability that a model is the “best” model in the candidate set), ER = evidence ratio (the odds ratio in favor of the “best” model over another), and LL = log likelihood.

Analysis	Model	K	AICc	ΔAICc	w_i	ER	LL
Call Frequency Classification	Class One	5	4892.26	0	0.96		-2441.03
	Class Two	4	4898.56	6.31	0.04	23.41	-2445.22
	Class Three	4	4943.84	51.58	0.0	1.58e11	-2467.86
	Class Four	3	4954.79	62.53	0.0	3.78e13	-2474.36

Table III-4. Each of the five hypothetical models ranked from best (top – lowest AICc) to worst (bottom – highest AICc) for each analysis. K = number of parameters, w_i = Akaike weight (probability that a model is the “best” model in the candidate set), ER = evidence ratio (the odds ratio in favor of the “best” model over another), and LL = log likelihood.

Analysis	Model	K	AICc	Δ AICc	w_i	ER	LL
Antipredator behavior	Call frequency	6	73.13	0	0.76		-30.26
	Species	6	76.22	3.09	0.16	4.7	-31.80
	Patry	4	77.79	4.66	0.07	10.3	-34.75
	Family	4	83.68	10.55	0	195	-37.70
	Genus	5	84.27	11.14	0	262	-36.92
Latency to Respond	Call Frequency	7	256.45	0	0.99		-120.81
	Species	7	266.32	9.87	0.01	139	-125.74
	Patry	5	269.42	12.97	0	657	-129.49
	Family	5	274.26	17.81	0	7380	-131.91
	Genus	6	276.07	19.63	0	18266	-131.73
Vigilance – During	Patry	4	202.30	0	0.46		-97.0
	Call frequency	6	203.38	1.09	0.27	1.7	-95.38
	Family	4	204.70	2.40	0.14	3.3	-98.20
	Species	6	205.82	3.52	0.08	5.8	-96.60
	Genus	5	206.64	4.34	0.05	8.8	-98.10
Vigilance – Two minute post	Patry	4	366.49	0	0.56		-179.10
	Species	6	367.91	1.42	0.28	2.04	-177.65
	Call frequency	6	369.04	2.55	0.16	3.57	-178.21
	Family	4	388.99	22.5	0.0	76881	-190.35
	Genus	5	389.29	22.8	0.0	89430	-189.43
Eye state – Two minute post	Patry	5	90.06	0	0.81		-39.69
	Species	7	94.63	4.57	0.08	9.8	-39.67
	Call frequency	7	95.49	5.44	0.05	15.1	-40.10
	Genus	6	96.75	6.69	0.03	28.4	-41.90
	Family	5	97.37	7.31	0.02	38.7	-43.35

Table III-5. Mean latency to respond of male *Anolis sagrei* to stimuli based on frequency classification. Frequency classification ($\chi^2 = 8.14$, DF = 4, $p = 0.087$) had a marginally non-significant effect on lizard response times.

Analysis	Frequency (Hz)	N	Mean $\pm$ SE	Range
Latency to Respond (sec)				
	Control	26	1.17 $\pm$ 0.32	0.03 – 7.18
	Low	29	2.64 $\pm$ 0.68	0.07 – 16.2
	Medium	25	2.25 $\pm$ 0.67	0.03 – 13.0
	Medium-High	44	1.80 $\pm$ 0.46	0.03 – 16.7
	High	8	3.13 $\pm$ 1.66	0.20 – 13.5

Table III-6. Number and proportion of lizards that did not respond or responded with antipredator behavior to playback stimuli. Frequency classification ($\chi^2 = 4.54$, DF = 4, $p = 0.34$) did not have a significant effect on the likelihood of whether lizards responded with antipredator behavior.

Frequency Classification	No Response	Antipredator Response	Proportion Responded
Control	26	2	0.0714
Low	28	1	0.0345
Medium	25	6	0.194
Medium-high	44	44	1.0
High	8	2	0.20

Table III-7. Parameter estimates from the ‘best’ model for each analysis. Effects that are significantly different from 0, based on 95%, CIs are shown in bold.

Analysis	Variable	Estimate	SE	Z value	P value
Antipredator Behavior	Intercept	2.83	0.825	3.43	0.0006
	High	-1.35	1.18	-1.16	0.248
	Low	0.838	1.29	0.648	0.517
	Medium	-1.20	0.937	-1.28	0.202
	Medium-high	17.99	2553	0.007	0.994
Latency to respond	Intercept	0.574	0.275	2.09	0.037
	High	1.08	0.428	2.52	0.012
	Low	0.144	0.293	0.492	0.623
	Medium	0.686	0.321	2.14	0.033
	Medium-high	-0.407	0.271	-1.50	0.133
Vigilance – During	Intercept	0.900	0.096	9.38	2e-16
	Control	-0.014	0.136	-0.102	0.919
	Sympatric	0.197	0.124	1.58	0.113
Vigilance – Two minute post	Intercept	0.731	0.173	4.22	2.42e-5
	Control	0.326	0.117	2.78	0.0055
	Sympatric	0.587	0.104	5.63	1.84e-8
Eye State	Intercept	3.12	0.172	18.2	2e-16
	Control	-0.046	0.272	-0.171	0.865
	Sympatric	0.760	0.272	2.80	0.005

Table III-8. Summary statistics for vigilance for the 20-second period during playback stimuli and the two-minute period after hearing playback stimuli. See Table III-9 for pairwise comparisons.

Analysis	Distance	N	Mean $\pm$ SE	Range
Vigilance – 20 second	Sympatric	29	3.17 ± 0.406	1 – 9
	Allopatric	85	2.59 ± 0.210	0 – 8
	Control	28	2.57 ± 0.315	0 – 6
Vigilance - Two minute	Sympatric	29	5.10 ± 1.03	0 – 20
	Allopatric	85	2.86 ± 0.360	0 – 16
	Control	28	3.79 ± 0.831	0 – 18

Table III-9. Pairwise comparisons conducted on the multi-level predictor variables that had significant 95% confidence intervals from the ‘best’ model in the candidate set for each analysis. Comparisons that are significantly different from one another, based on 95% CIs, are shown in bold.

Analysis	Comparison	Estimate	SE	Z value	P value	95% CI
Vigilance – Two minute post	Allopatric – Control	-0.325	0.117	-2.78	0.015	-0.600 – -0.0507
	Allopatric – Sympatric	-0.587	0.104	-5.63	<0.0001	-0.831 – -0.342
	Control – Sympatric	-0.261	0.128	-2.04	0.102	-0.561 – 0.0385
Eye State	Allopatric – Control	0.0464	0.272	0.171	0.984	-0.592 – 0.684
	Allopatric – Sympatric	-0.760	0.272	-2.80	0.0142	-1.40 – -0.124
	Control – Sympatric	-0.806	0.333	-2.42	0.0413	-1.59 – -0.0250

Table III-10. Duration (seconds) of time spent with eyes open for the two-minute period following playback of the stimuli. Familiarity ($\chi^2 = 8.63$, $df = 2$, $p = 0.013$) had a significant effect on lizard 'alertness'.

Familiarity Grouping	N	Mean $\pm$ SE	Range
Sympatric	19	55.8 ± 9.7	10 – 90.14
Allopatric	57	25.9 ± 3.1	0 – 120.0
Control	19	25.0 ± 5.1	4.03 – 120.0

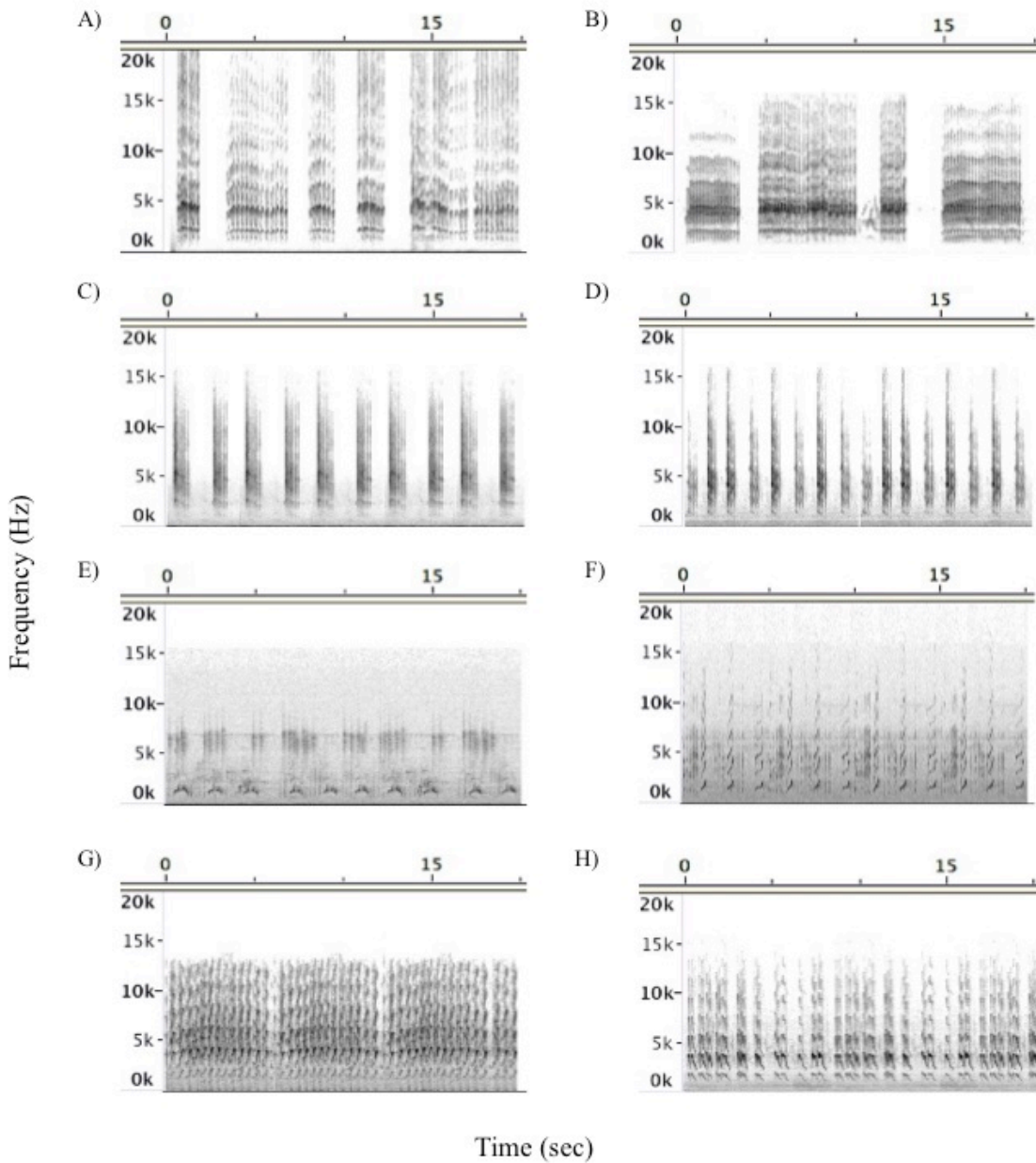


Figure III-1. Spectrograms of each of the eight exemplars (two exemplars from four species) used for testing. Spectrograms are arranged in order of phylogenetic distance with respect to the sympatric predator, from most closely related to most distantly related. A) *Falco sparverius* 1 B) *F. sparverius* 2 C) *F. naumanni* 1 D) *F. naumanni* 2 E) *Poliheirax insignis* 1 F) *P. insignis* 2 G) *Accipiter badius* 1 H) *A. badius* 2

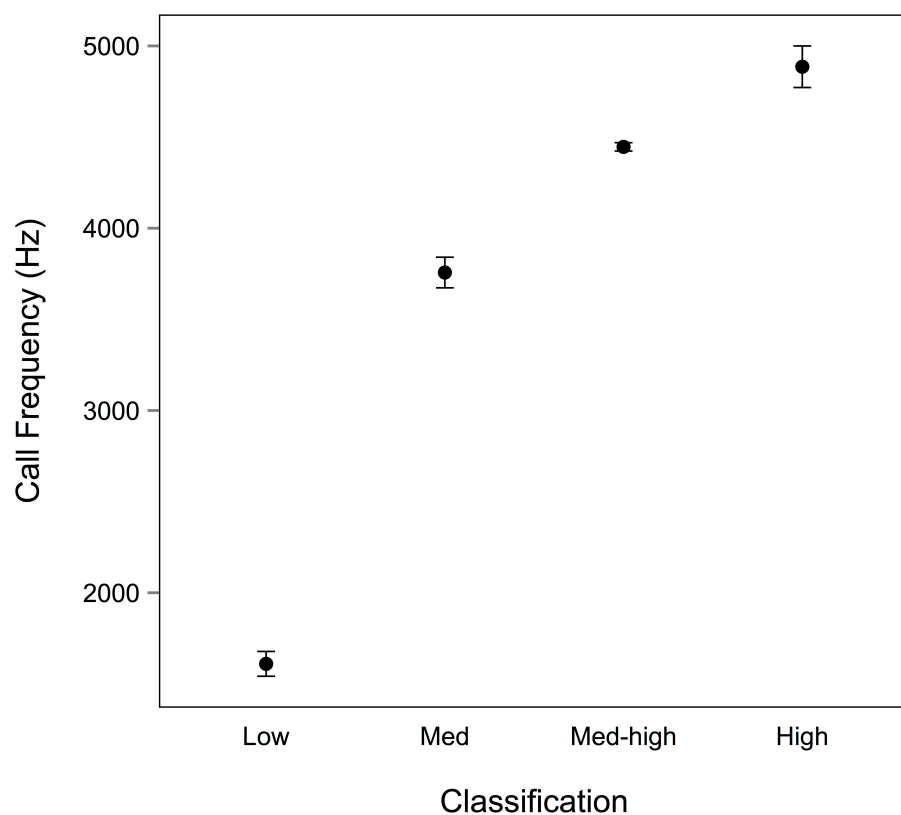


Figure III-2. Average call frequency ($\pm$ SE) of the four classifications from the best model.

‘Low’ classification included both *P. insignus* exmplars, ‘Medium’ included *F. naumanni* 2 and *A. badius* 2, ‘Medium-High’ included both *F. sparverius* exemplars and one *A. badius* exemplar, and ‘High’ included one *F. naumanni* exemplar. See Table III-2 for summary statistics of frequency classifications.

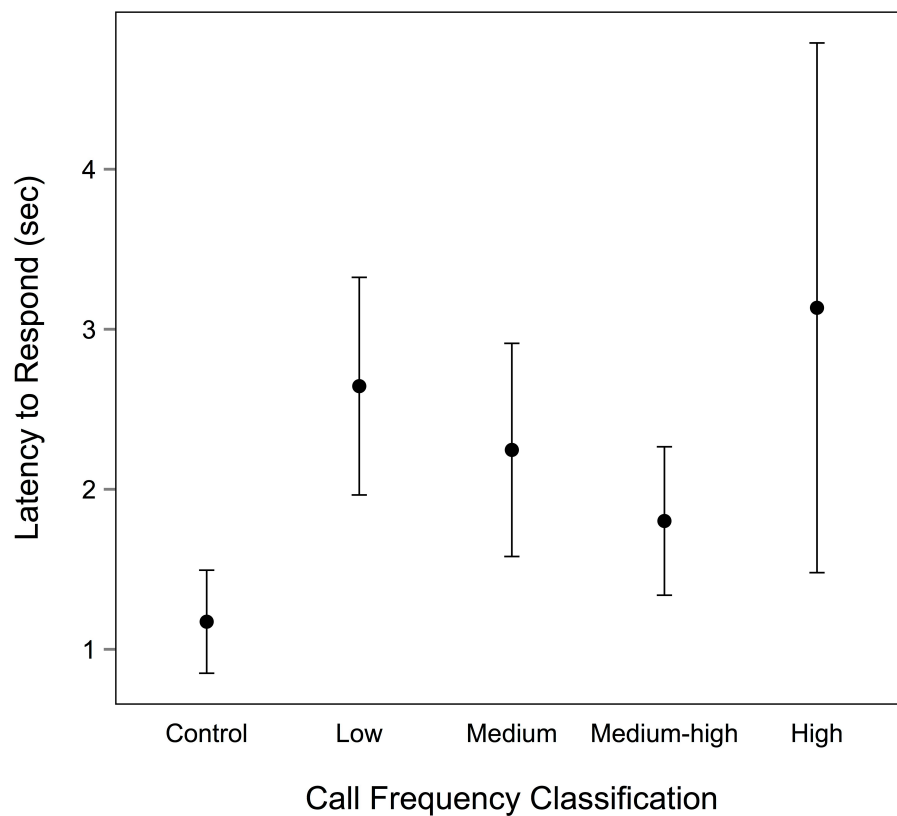


Figure III-3. Mean latency to respond ($\pm$ SE) to the various stimuli based on frequency classifications. Frequency classification had a marginally non-significant effect on lizard response times ($\chi^2 = 8.14$, $DF = 4$, $p = 0.087$).

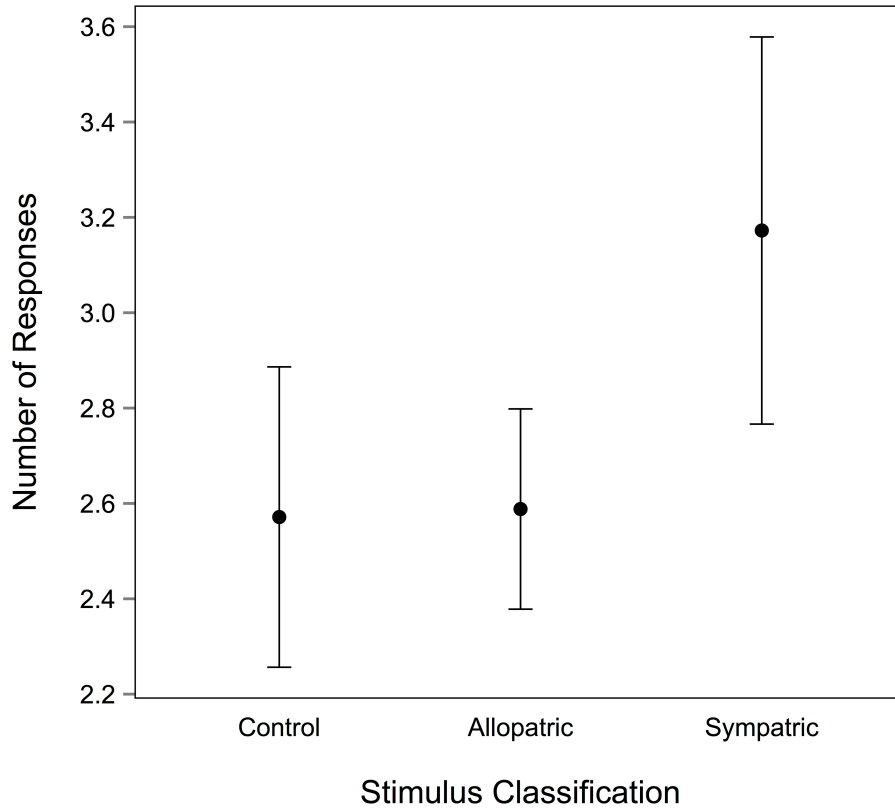


Figure III-4. Average number of antipredator responses elicited by sympatric and allopatric predator vocalizations and control stimuli. 'Patry' did not have an effect on the number of antipredator responses ($\chi^2 = 2.81$, $df = 2$, $p = 0.246$). Playbacks of the sympatric predator calls elicited the greatest number of antipredator behaviors in lizards compared to allopatric predator calls and the control.

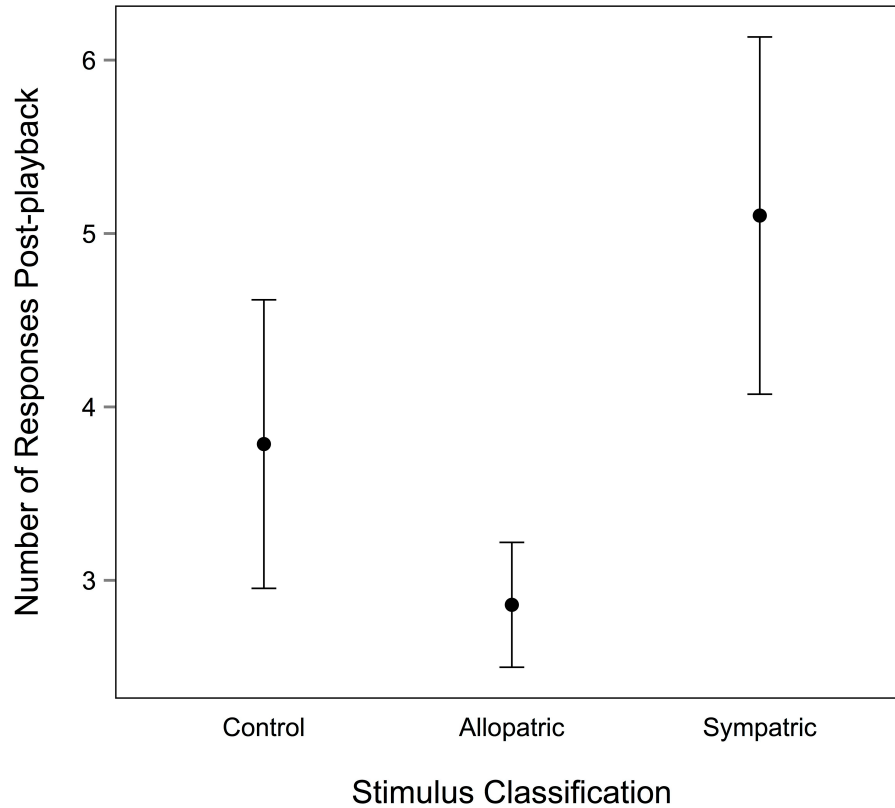


Figure III-5. Average number of antipredator responses exhibited during the two-minute period following playback of stimuli. ‘Patry’ had a significant effect on antipredator behavior ($\chi^2 = 32.5$, $df = 2$, $p = 8.7e-08$). Anoles exhibited significantly more responses after hearing playbacks of the sympatric predator calls compared to allopatric predator calls (95% CI -0.831 – -0.342). In contrast, anoles were significantly less vigilant after hearing allopatric calls compared to the control (95% CI -0.600 – -0.507). There was marginally non-significant difference in vigilance between sympatric calls and the control (95% CI -0.561 – 0.0385).

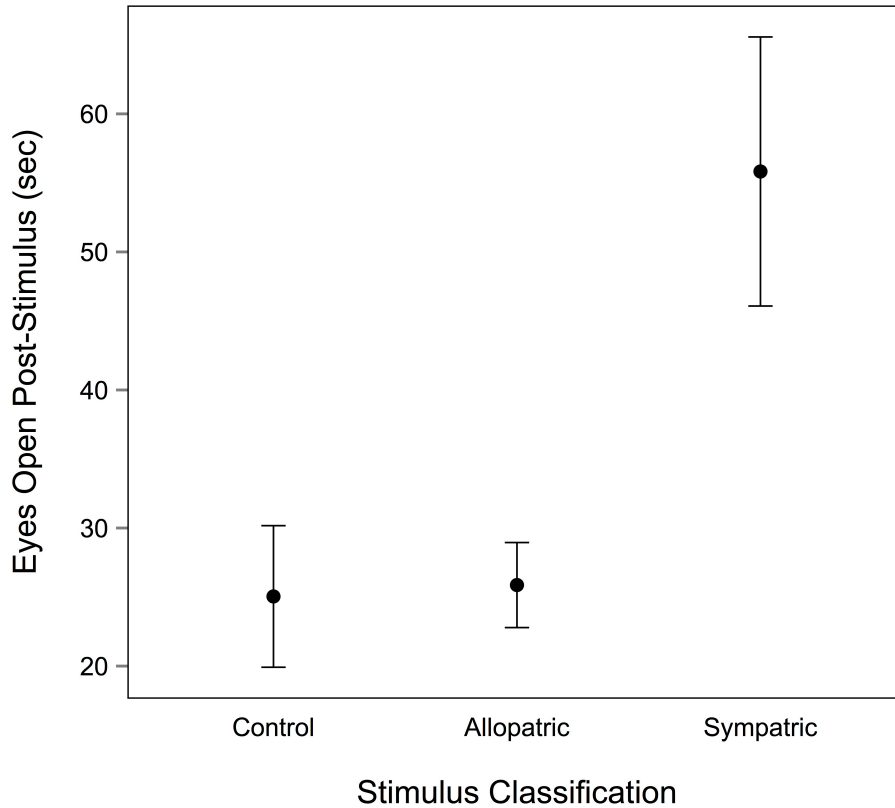


Figure III-6. The average amount of time that lizards remained alert during the two-minute period following playback of sympatric and allopatric predator vocalizations and control stimuli. ‘Patry’ had a significant effect on ‘alertness’ ($\chi^2 = 8.63$, $df = 2$, $p = 0.013$). Anoles were significantly more alert after hearing the sympatric predator call compared to the allopatric predator calls (95% CI -1.40 – -0.124) and the control (95% CI -1.59 – -0.0250). There was no difference in lizard ‘alertness’ after hearing the allopatric calls compared to the control (95% CI -0.592 – 0.684).

CONCLUSIONS

Predation is a significant factor driving the evolution of both morphological and behavioral traits in animals. Importantly, animals that are able to recognize predators in the early stages of a predatory encounter, or even before an encounter occurs, are more likely to increase their chances of survival, and ultimately increase their fitness (Caro 2005; Lima and Dill 1990; Pianka and Vitt 2003). Mounting evidence has shown the importance of various sensory (visual, chemical, and auditory) cues (Clucas et al. 2010; Hettner et al. 2014; Vandyk and Evans 2007; Webb et al. 2009) and predator characteristics (Stankowich and Blumstein 2005) in alerting prey to danger. Moreover, prey that are able to incorporate information about predators into decision making are more likely to reduce their predation risk (Caro 2005; Cooper 2008; Stankowich and Coss 2006).

Most animals do not vocalize while hunting. However, it is important for prey to recognize when a predator is active in the environment, as predation events can be unpredictable and occur at any given time. Auditory cues can alert prey to danger prior to a predatory encounter, thereby providing them with the opportunity to reduce their risk of predation. The research conducted for this dissertation addressed the ability of brown anoles (*Anolis sagrei*) to use auditory cues to assess predation risk. Specifically, I investigated 1) whether male and female brown anoles can discriminate between the calls of predatory and non-predatory birds and if they differ in their antipredator responses, 2) the importance of sound pressure level in determining predator proximity, and 3) the underlying proximate mechanisms used to recognize auditory predator cues.

The results from Chapter I add to the growing body of evidence that non-vocal lizards use auditory cues to assess risk and that they are able to distinguish between threatening and non-

threatening cues (Cantwell and Forrest 2013; Elmasri et al. 2012; Fuong et al. 2014; Huang et al. 2011; Ito et al. 2013; Ito and Mori 2010; Vitousek et al. 2007). Male lizards were significantly more apt to increase vigilance behavior during and after hearing predator vocalizations. Furthermore, female lizards were more skittish, in general. Testing was conducted during the peak of breeding season, therefore, females were likely gravid. Excessive wariness in females is likely beneficial during breeding season due to higher probability of survival and increased fitness.

Data from Chapter II provide mixed results on the importance of sound pressure level for assessing predator proximity. Lizards significantly increased vigilance during playbacks of vocalizations played from a distance of eight meters and after hearing vocalizations played from a distance of four meters. The lack of increased vigilance to playbacks from very near distances may be the result of freezing behavior, however, further studies are needed to address this possibility. In addition, conspicuous courtship displays were reduced after hearing vocalizations from all distances, suggesting that a predator in the general vicinity may be enough for prey to modify risky/conspicuous behavior.

Finally, results from Chapter III provide evidence that lizards may require some degree of familiarity with calls to be able to recognize predatory vocalizations as dangerous. Conversely, it also possible that lizards generalize antipredator responses to calls with similar auditory characteristics, such as call frequency. The top two models for all analyses were those in support of familiarity or call frequency. While lizards did not differ in the time it took to respond to playbacks or differentially increase vigilance during playbacks, they were twice as alert after hearing playbacks of the sympatric predator call compared to the other stimuli. It is highly

possible that the semi-anechoic chambers that the lizards were tested in created such an unnatural silence that the environment in and of itself was considered to be threatening.

Summary

Combined, these results provide evidence that auditory cues are important for non-vocal lizards to assess predation risk. Data collected also point to some possible environmental factors affecting antipredator responses including perch height, wind speed, and canopy cover; however the role and the extent to which these played a part in altering responses is less understood without further testing. Furthermore, call frequency may help to explain the lack of differences found in response times to the various stimuli used throughout testing.

Future Directions

While I have documented the importance of auditory cues for assessing predation in non-vocal lizards, many questions remain unanswered. Investigation into the ability of lizards to use auditory cues only recently emerged (within the last decade); consequently, relatively few species have been tested. Expanding studies to include a wider taxonomic base (i.e. families, genera, species, etc.) will inevitably provide an answer to how widespread this ability is but may also help to explain how and why the auditory system evolved over time. In other words, did the auditory system evolve to assist with communication or assess surroundings? Future studies should also consider investigating the role of the sympathetic nervous system on physiological responses such as stress hormones, heart rate and respiration rate, in response to auditory distance cues associated with threatening stimuli. Last, to further understand whether responses are innate or learned, studies should be conducted on naïve individuals that have never experienced bird vocalizations and other auditory sounds.

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VITA

Lisa Ragan Cantwell was born in Asheville, NC. After graduating high school, she took time off from school and worked various jobs in the customer service industry until finding her true calling, working with animals and helping others. She began a job as a veterinary assistant at a local veterinary hospital in 2000 where she spent the next 10 years working while also attending school. She received her Associate in Arts degree in May 2006 from Asheville-Buncombe Technical Community College followed by her Bachelor of Science in Biology from the University of North Carolina Asheville in December of 2009. Lisa began graduate school in 2010 at the University of Tennessee at Knoxville in the Department of Ecology and Evolutionary Biology studying reptile behavior and graduated with a Doctor of Philosophy degree in Spring 2016.