**The Dissertation Committee for Patricia Lucile Jones Certifies that this is the approved version of the following dissertation:**

# Foraging Decisions by Eavesdropping Bats

**Committee:**

Michael J. Ryan, Supervisor

Rachel A. Page, Co-Supervisor

Lawrence E. Gilbert

Molly E. Cummings

Ulrich G. Mueller

# Foraging Decisions by Eavesdropping Bats

**by**

**Patricia Lucile Jones, B.A.**

## Dissertation

Presented to the Faculty of the Graduate School of
The University of Texas at Austin
in Partial Fulfillment
of the Requirements
for the Degree of

**Doctor of Philosophy**

**The University of Texas at Austin**
**May, 2014**

# Dedication

To my parents and my sister, I love you.

To my grandmother, Pat Fouraker, I miss you.

# Acknowledgements

There are many people who made this possible. First, thank you to my two wonderful supervisors Mike Ryan and Rachel Page for your support, encouragement, and advice throughout. I am deeply grateful. Thank you also to all the members of my committee for their helpful feedback: Ulrich Mueller, Molly Cummings and Larry Gilbert. I also feel very lucky to have been able to work with two amazing biologists who recently passed away: Björn Siemers and Elizabeth Kalko. You have been an inspiration to me and I wish I could have had more time to learn from you.

Thank you to all who helped me in the field: Victoria Flores, Teague O'Mara, Tess Driessens, Jay Falk, Christina Buelow, Sarah Richman, May Dixon, Kristina Ottens, and Teia Schwietzer. I am very grateful for your good company, late night carrot cake, trips to the beach, and lots of laughter. You kept me sane! Thank you to all of the wonderful members of the Ryan Lab who have provided a happy atmosphere and given me good advice: Pam Willis, Karin Akre, Monica Guerra, Sofia Rodriguez, Audrey Stewart, Meghan Still, Bret Pasch, and Heidi Smith Parker. You are all fantastic scientists and great people. I will miss working with you.

I also thank my roommates over the years in Austin: Laura Crothers, Bonnie Waring, Kelly Pierce and their respective felines. Thank you for being so supportive and making our house home. To the members, past and present, of the Austin Rowing Club's Women's Competitive Team, thank you for all the workouts, the races, and the breakfast tacos. You have been an essential retreat for me and I have loved rowing with you.

And to my family, Mom, Dad and Lee. I could not have made it this far without your love and support and I can never thank you enough.

# Foraging Decisions by Eavesdropping Bats

Patricia Lucile Jones, Ph.D.
The University of Texas at Austin, 2014

Supervisors: Michael J. Ryan and Rachel A. Page

Animals forage in complex environments in which they must constantly make decisions about which resources to approach and which to avoid. Many factors can influence these foraging decisions including perception and cognition. Predators that locate prey by eavesdropping on prey mating calls face a challenging foraging task because they must be able to identify which species-specific prey signals indicate palatable prey. My thesis investigates such foraging decisions in eavesdropping bats. The Neotropical fringe-lipped bat, *Trachops cirrhosus*, locates its frog and katydid prey by eavesdropping on the prey's calls. One of the prey of *T. cirrhosus* in Panamá is the túngara frog, *Physalaemus pustulosus*, that can make simple calls consisting of a "whine" alone, or complex calls which are a whine followed by 1-7 "chucks". In my first chapter I examine what components of frog calls bats use to identify and localize them. I assess how bats respond to the two components of the complex calls of *P. pustulosus*, and report that, unlike female frogs, bats respond to the chuck component alone but preferentially approach the whine. Next, I examine how response to prey cues is affected by prey availability by assessing the response of *T. cirrhosus* to geographically and seasonally variable prey. I find population and seasonal differences in response to some prey cues but not to other cues. *Trachops cirrhosus* can also learn novel prey cues from exposure to a conspecific tutor (social learning). My third chapter examines the conditions that influence when bats socially learn novel prey cues. I discover that bats are more likely to use social information to learn novel prey cues when the cue they are currently using to find food is unreliable. In my fourth and final chapter I address how eavesdropping can contribute to the evolution and diversification of bats by investigating the potential of eavesdropping on katydid calls for niche partitioning in two closely related bat species, the European greater and lesser mouse-eared bats, *Myotis myotis* and *Myotis blythii oxygnathus*. Together these studies highlight the role of cognition in foraging decisions and consider the consequences of eavesdropping for niche partitioning.

# Table of Contents

# List of Tables

# List of Figures

# INTRODUCTION

“I want to know what it is like for a bat to be a bat. Yet if I try to imagine this, I am restricted to the resources of my own mind, and those resources are inadequate to the task…Even if I could by gradual degrees be transformed into a bat, nothing in my present constitution enables me to imagine what the experiences of such a future stage of myself thus metamorphosed would be like. The best evidence would come from the experiences of bats, if we only knew what they were like” (Nagel 1974).

## BACKGROUND

The study of foraging behavior underwent a revolution in the late 1960’s with the development of optimal foraging theory (Emlen 1966; MacArthur and Pianka 1966). Optimal foraging theory transformed the examination of foraging from descriptive accounts of what animals were eating to quantitative assessments and predictions centered on why animals were making certain foraging decisions, and produced decades of fruitful research (*reviewed in* Stephens et al. 2007). Since the 1980’s there has been another important development in the inclusion of literature on learning, psychology, and neurobiology in the study of foraging. The role of learning and memory in foraging decisions have been investigated most thoroughly for honeybees (*reviewed in* Sherry & Mitchell 2007) and for corvids (*reviewed in* Adams-Hunt & Jacobs 2007) for which laboratory studies have illuminated many of the cognitive factors that influence foraging decisions.

What remains poorly developed, however, is the integration of laboratory studies of cognition with the decisions animals have to make in the wild, and the evolutionary consequences of such decisions. In the chapters that follow I address these questions in bats that hunt their prey by eavesdropping on prey mating calls. Eavesdropping is defined as "the use of information in signals by individuals other than the primary target" (Peake 2005), and can be an important selective force on the mate-attraction signals of prey (Zuk and Kolluru 1998). Eavesdropping may also influence the evolution of auditory capabilities (Bruns and Burda 1989; Robert et al. 1992), ecology (Tuttle et al. 1985), and foraging behavior (Page and Ryan 2005) of predators. Eavesdropping is an unusual type of foraging behavior because predators associate prey with species-specific signals. Predators must be able to determine which signals indicate palatable prey and which are from poisonous or otherwise inedible prey that should be ignored or avoided. I propose that eavesdropping is a foraging strategy in which cognition could play an important role in the form of learning new prey signals and flexibly shifting responses with environmental variability.

Eavesdropping on prey cues has been described for multiple species of bats from different families (Buchler and Childs 1981; Tuttle and Ryan 1981; Spangler 1984; Tuttle et al. 1985; Belwood and Morris 1987; Ryan and Tuttle 1987; Bailey and Haythornthwaite 1998; Hofstede et al. 2008). One of the study systems for my research is the fringe-lipped bat or frog-eating bat, *Trachops cirrhosus*, that hunts frogs by approaching their calls (Tuttle and Ryan 1981). *Trachops cirrhosus* also eavesdrops on the calls of katydids, but preferentially approaches frog calls over katydid calls (Tuttle et al. 1985). *Trachops cirrhosus* in the area surrounding the Panama Canal respond to the calls of palatable frog species, but not to the calls of poisonous toads (Tuttle and Ryan 1981) (Figure 1). *Trachops cirrhosus* also generalize their responses to include similar-sounding novel calls (Ryan and Tuttle 1983), and can very quickly learn novel associations between prey cues and prey quality both through individual learning (Page and Ryan 2005), and social learning (Page 2006; Jones et al. 2013). The capability of *T. cirrhosus* to learn novel prey cues may be a solution to the challenge posed by eavesdropping as a foraging strategy for a generalist predator. The first three chapters of my thesis focus on cognitive ecology in *T. cirrhosus*. In particular, I examined the factors that affect which prey foraging bats select to attack.

**Figure 1:** The fringe-lipped or frog-eating bat and a túngara frog. Photo by Christian Ziegler.

As a means of locating particular prey, eavesdropping can also enable niche partitioning between closely related species. For example, passive listening for prey-generated sounds versus active prey localization through echolocation appears to partition niches between the closely related European species *Myotis bechstennii* and *M. nattereri*. *M. bechstennii* relies more on prey-generated cues while *M. nattereri* relies more on echolocation to locate prey, enabling the two species to access different types of prey (Siemers and Swift 2006). Also, *T. cirrhosus* and one of its closely related species, *Lophosoma sylvicola*, are both eavesdropping bats but respond differently to frog and katydid calls (Tuttle et al. 1985). The means by which predators locate prey may thereby enable species divergence. My final chapter examines the potential role of eavesdropping in niche partitioning between European greater and lesser mouse-eared bats, *Myotis myotis* and *Myotis blythii oxygnathus*, which are morphologically similar sister species.

## RESEARCH QUESTIONS

**Chapter 1.** Female *P. pustulosus* frogs and frog-eating bats, *T. cirrhosus,* exhibit phonotaxis to the calls of male *P. pustulosus* frogs (Tuttle and Ryan 1981; Ryan 1985). Female frogs do not respond to the chuck component of the call when it is broadcast alone, but when a whine is also broadcast, spatially separated from the chuck, frogs preferentially approach the chuck. I examined how bats perceive and localize the different components of complex frog calls, and compared these responses to what is already known about perception and localization in female frogs (Farris et al. 2002). Bats and frogs have converged to respond to the same signal, but they do so using very different neural and cognitive architecture.

**Chapter 2.** Due to their reliance on species-specific signals, eavesdropping predators may be particularly sensitive to variation in available prey. I examined population and seasonal differences in how *T. cirrhosus* responds to prey cues. Bats can quickly learn to associate novel prey cues with food rewards (Page and Ryan 2005). I hypothesized that this learning capability enables bats to alter their foraging behavior to take advantage of seasonal prey, and predicted that bats would be most responsive to prey that is currently available.

**Chapter 3.** Not only do *T. cirrhosus* quickly learn novel prey cues individually, they can also learn novel prey cues from interactions with knowledgeable conspecifics, or social learning. Bats actually learn novel associations through social learning much faster than through individual trial and error learning (Page and Ryan 2006). I investigated the conditions that influence when bats learn novel prey cues by social learning.

**Chapter 4.** Species differences in diet have been demonstrated for the European greater and lesser mouse-eared bats, *Myotis myotis* and *M. blythii oxygnathus* (Arlettaz et al. 1997) (Figure 2) that are morphologically very similar and roost together in the same caves. *M. myotis* predominantly eats carabid beetles which is locates using the rustling sounds of beetles moving through leaf-litter (Russo et al. 2007), and *M. b. oxygnathus* predominantly eats katydids but it is unknown how they locate them. I examined whether *M. b. oxygnathus* locates katydids by eavesdropping on their calling songs and whether there are species differences in how these sister taxa respond to prey cues.

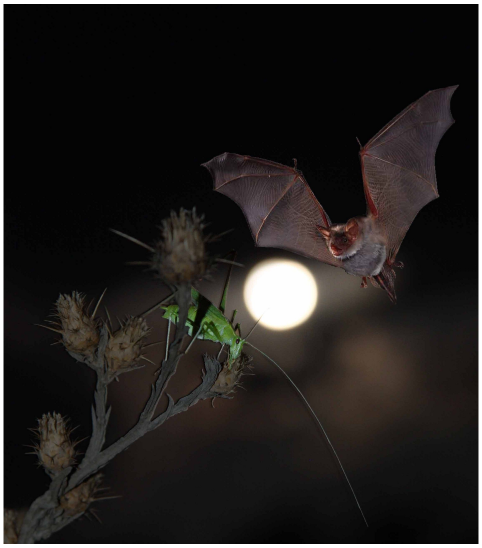

**Figure 2:** A lesser mouse-eared bat approaches a katydid. Photo by Björn Siemers.

# CHAPTER 1

## Do frog-eating bats perceptually bind the complex components of frog calls?[1]

### ABSTRACT

The mating calls of male túngara frogs, *Physalaemus pustulosus*, attract intended (conspecific females) and unintended (eavesdropping predators and parasites) receivers. The calls are complex, having two components: a frequency modulated "whine" followed by 0-7 harmonic bursts or "chucks". The whine is necessary and sufficient to elicit phonotaxis from females and the chuck enhances call attractiveness when it follows a whine. Although chucks are never made alone, females perceptually bind the whine and chuck over spatial separation. We tested whether an unintended receiver with independent evolution of phonotaxis, the frog-eating bat, *Trachops cirrhosus*, has converged with frogs in its auditory grouping of the call components. In contrast to frogs: bats approached chucks broadcast alone; when the chuck was spatially separated from the whine the bats preferentially approached the whine; and bats were sensitive to whine-chuck temporal sequence. This contrast suggests that although disparate taxa may be selected to respond to the same signals, different evolutionary histories, selective regimes, and neural and cognitive architectures may result in different weighting and grouping of signal components between generalist predators and conspecific mates.

---

[1] Published as: Jones PL, Farris HE, Ryan MJ, Page RA (2013) Do frog-eating bats perceptually bind the complex components of frog calls? *Journal of Comparative Physiology A* 199:279-283. Author Contributions: Jones designed the experiment, conducted the research and wrote the manuscript. Farris, Ryan and Page provided advice on experimental design and edited the manuscript.

## INTRODUCTION

Conspicuous sexual advertisement signals attract potential mates (Darwin 1871; Andersson 1994), but may also attract eavesdropping predators or parasitoids (Zuk and Kolluru 1998; Peake 2005; Jones et al. 2011, Page et al. *in press*). Indeed, conspecific mates and heterospecific eavesdroppers use the same signals to identify, locate, and assess the quality of the signaler (Walker 1993, Wagner 1996, Haynes and Yeargen 1999, Bernal et al. 2006). The extent to which signal processing converges in these intended and unintended receivers may depend on how specialized their phonotaxis is to a particular signal. For example, specialized dipteran parasitoids of a single singing insect species exhibit similar auditory tuning and phonotactic preferences to those of mate-searching females (Fowler 1987; Robert et al. 1992; Walker 1993; Wagner 1996; Lakes-Harlan et al. 1999; Gray et al. 2007, Farris et al. 2008; Wagner 2011). In contrast, generalist parasitoids may exhibit less convergence with females of the host species (Stumpner et al. 2007; Sakaguchi and Gray 2011). Our study investigates whether a generalist acoustic predator, the frog-eating bat (*Trachops cirrhosus*), groups the complex call components of male túngara frogs (*Physalaemus(=Engystomops) pustulosus*) as female túngara frogs do.

Calls of túngara frogs have two acoustically distinct components: a frequency-modulated ~350 ms sweep (“whine”) and a broadband ~40-80 ms harmonic burst (“chuck”) (Ryan 1980). Males can produce simple calls consisting of a whine alone, or complex calls composed of a whine followed by 1-7 chucks. Both female frogs and frog-eating bats are more attracted to complex calls than simple calls (Ryan 1980; Ryan et al. 1982, Akre et al. 2011). Male frogs call in multi-male choruses creating a problem for female frogs and frog-eating bats that is acoustically analogous to the ‘cocktail party problem’ in humans (Cherry 1953). Female frogs and bats must determine which whine goes with which chuck, so that calls can be assigned to the correct source and thereby accurately compared. In female frogs the whine is necessary and sufficient to elicit phonotaxis, but frogs exhibit a conditional response to the chuck: a chuck that elicits no response when presented alone is attractive and elicits phonotaxis when broadcast with the spatially separated whine (Ryan 1985; Farris et al. 2002). This conditional phonotactic response reveals auditory grouping and source assignment of the two components that is based on relative whine-chuck spatial separation and temporal sequence (Farris et al. 2002; 2005; Farris and Ryan 2011). We tested whether this grouping response found in female frogs is also exhibited by frog-eating bats that are generalist acoustic predators of several frog species (Tuttle and Ryan 1981). The results allow us to compare the weighting and grouping of complex call components by two receivers that have different evolutionary histories and are under different selective regimes in their response to the same signal.

## METHODS

We captured bats with mist-nets in Soberanía National Park, Panamá between February and July of 2012 (N = 10: 7 adult males and 3 adult non-reproductive females).

Bats were released into a 5m x 5m x 2.5m flight cage with ambient temperature and humidity, illuminated by one 25W red light bulb. Only one bat was tested at a time. We placed Fostex FE103En speakers underneath 1.5m x 1.5m screens covered in leaf-litter in two diagonally opposite corners of the cage. In the third corner we positioned a shelter with a perch to which the bats were trained to return between stimulus presentations. The experimenters sat in the fourth corner with the playback equipment (see Page and Ryan 2005; 2006). The experimental stimuli were constructed in Adobe Audition 3 from the modal túngara frog call selected from a sample of 300 calls from 50 males (Ryan and Rand 2003). Stimulus period was 2 s and stimuli were broadcast at 75 dB SPL (re. 20 $\mu$Pa) at 1m from the speaker, reflecting natural call rate and amplitude (Rand and Ryan 1981; Ryan 1985). We broadcast stimuli using a Pyle Pro PTA2 amplifier and a Lenovo T500 Thinkpad laptop.

Each bat received six different stimuli (Fig. 3) four times each, presented in random order (24 presentations total). The whine (W) alone is sufficient to elicit phonotaxis in *T. cirrhosus* (Ryan et al. 1982). To assess whether the chuck (C) alone also elicits phonotaxis, we examined bat response in single speaker tests of either a single chuck (1C) or three consecutive chucks (3C). The 3C stimulus had a similar duration to the whine and was included in the design *a priori* in case bats were not responsive to a single chuck due to its short duration. The other four stimuli were broadcast with two speaker tests to determine how bats weight and group the two call components. For two of the stimuli, spatially separated whines and chucks were broadcast from the separate speakers in the two corners of the cage either in the natural (W vs. C) or reversed temporal order (C vs. W). These stimuli tested the relative weighting of the two components during phonotactic decisions and the extent that the natural temporal sequence affected such weighting. Previous research demonstrates that bats and frogs preferentially approach complex calls with higher ratios of chucks (Akre et al. 2011), indicating the importance of chucks in phonotactic decisions. We therefore also examined whether the chuck's influence on the whine's attractiveness was maintained even when presented without a co-localized whine. Thus, for the fifth stimulus, a whine was broadcast from one speaker followed by the chuck from both speakers (WC vs. C). The sixth stimulus reversed the temporal sequence to assess whether such a comparison is order-dependent (CW vs. C).

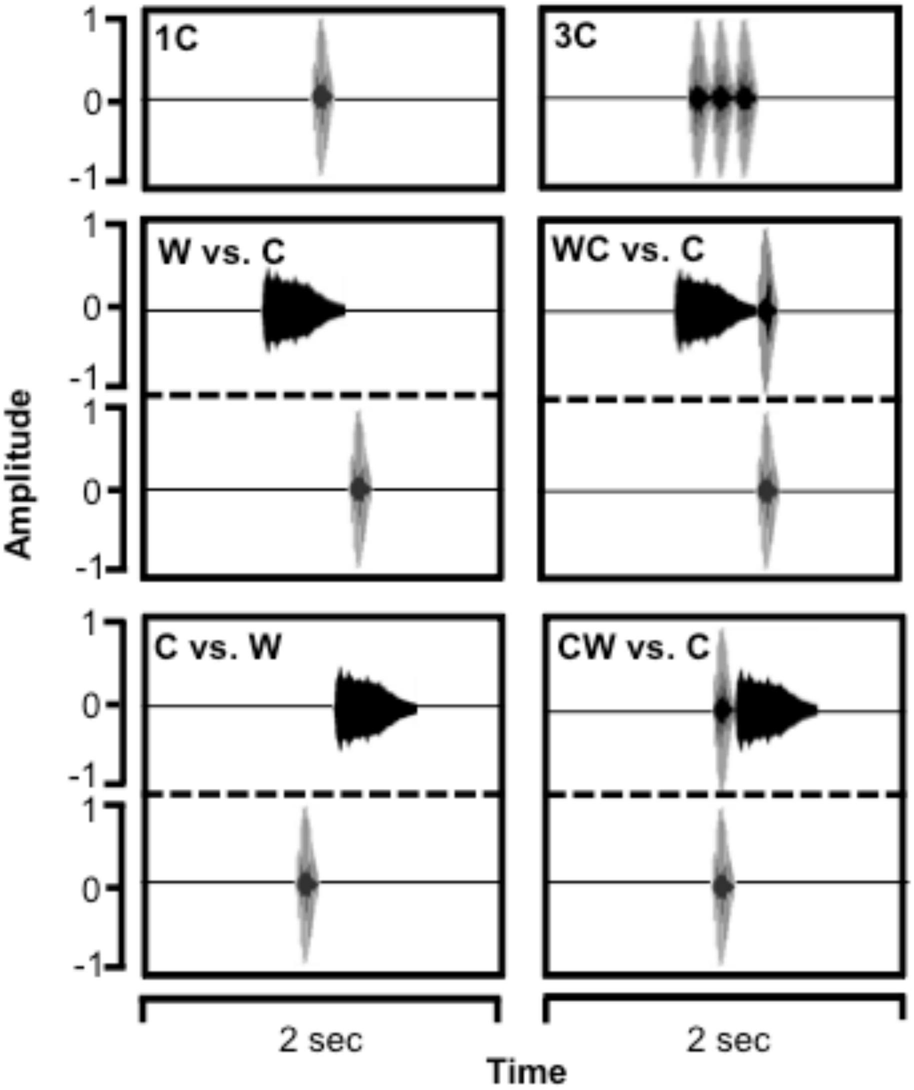

**Figure 3:** Waveforms of experimental stimuli. Dotted lines separate stimuli from two-speaker experiments.

1C, a single chuck from single speaker. 3C, three chucks from a single speaker. W vs. C, whine from one speaker, the chuck from the other in natural temporal sequence. WC vs. C, whine-chuck from one speaker and the identical chuck from the other speaker in natural temporal sequence. C vs. W and CW vs. C, as with the stimuli above except in reversed temporal sequence.

To control for arena bias the speaker side associated with the chuck component was randomly assigned for each stimulus. To maintain the bats' motivation, baitfish rewards were placed on the screens over both speakers. Tests lasted 20 sec or until the bat removed the baitfish from the speaker. Observers recorded which of the two speakers the bat approached for each stimulus. Analysis was conducted in R v. 2.15 (R Development Core Team 2012) and evaluated whether the number of times bats approached each of the stimuli (each bat had a value between 0 and 4 for each stimulus) differed from 0 (0%) in one speaker tests (did not approach the chuck) and 2 (50%) in two speaker tests (no preference). Significance was determined using one sample t-tests for each of the stimuli.

## RESULTS

Bats showed consistency in their responses across the four presentations. All ten individuals approached both the single chuck (1C) and the three chuck (3C) stimuli in at least two of the four presentations. The number of times that the bats approached both the 1C (one sample t-test: $t = 19$, $df = 9$, $p<0.0001$; Fig 4a) and the 3C significantly differed from zero ($t = 24$, $df = 9$, $p<0.0001$; Fig 4b). Bats therefore showed no conditional response to the chuck; they were attracted to chucks in the absence of whines even though this stimulus does not occur in the wild. We therefore used the two-speaker tests to determine if bats weight whines and chucks equally during phonotaxis.

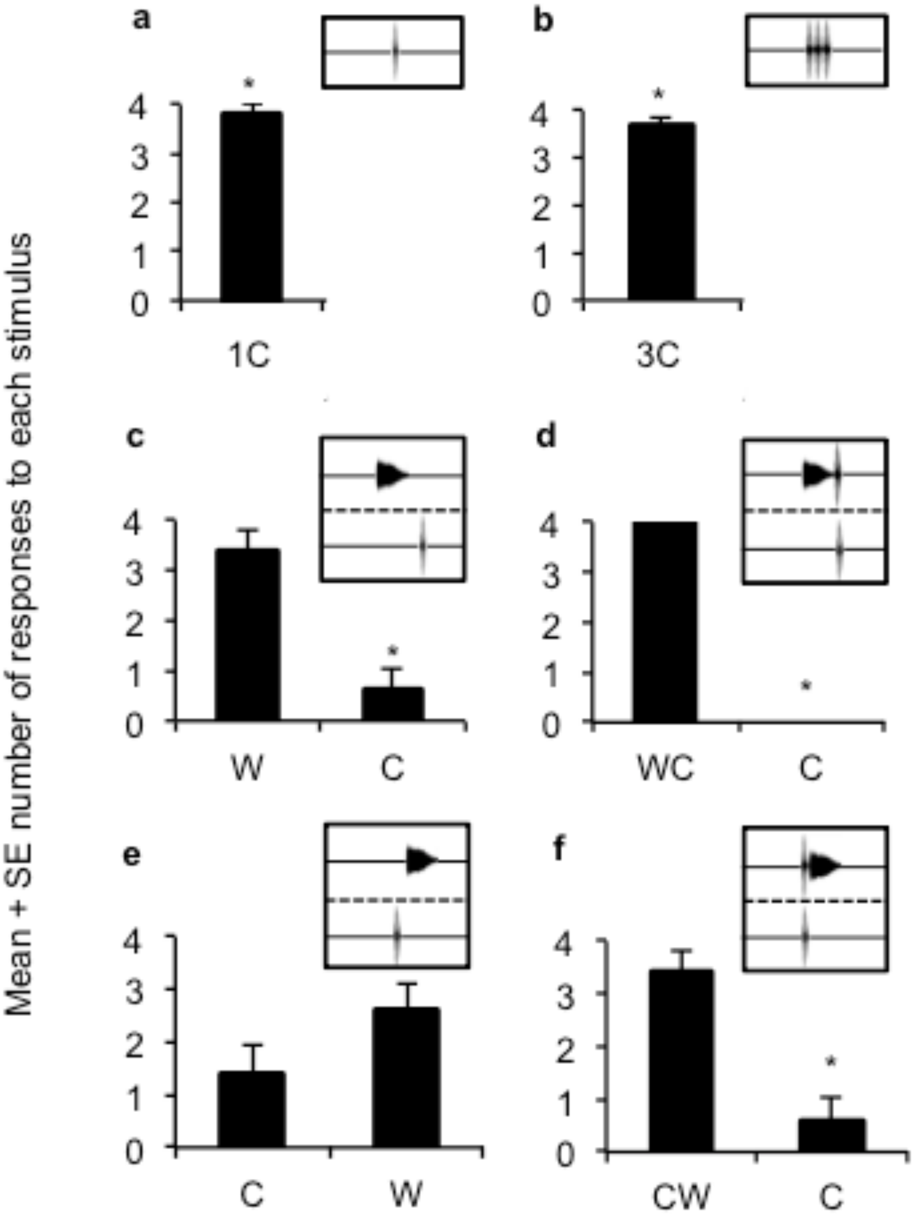

**Figure 4:** Mean (+SE) number of presentations for which bats approached each of the stimuli.

Each bat was tested 4 times for each stimulus. Stimuli are noted on the x-axis under the bars and insets are time waveforms. Asterisks indicate when the number of times bats approached the chuck significantly ($p<0.01$) differed from 0 (0%; one speaker tests) or 2 (50%; two-speaker tests).

When presented broadcasts of a spatially separated whine and chuck that maintained natural temporal sequence (W vs. C) bats approached the whine significantly more often than would be expected if they had no preference ($t = 3.5$, $df = 9$, $p = 0.007$; Fig 4c). These results contrast the conditional response to the chuck found in frogs. Bats are known to discriminate calls that have identical whines, but differ in their chucks (Akre et al. 2011). When chucks were controlled in the whine-chuck versus chuck tests (WC vs. C), however, all of the bats preferentially approached the whine-chuck for all of the presentations, showing that decisions were not based on chuck number alone and confirming that the whine plays an important role in phonotactic response (one-sample t-test: $t = 199$, $df = 9$, $p < 0.0001$; Fig 4d).

Bats' phonotactic preference for the whine was, in part, based on component sequence. When the call component order was reversed (C vs. W) bats did not approach the whine significantly more often than would be expected if they had no preference ($t = 1.2$, $df = 9$, $p = 0.26$; Fig 4e). The reverse order did not switch the preference to the chuck, however, suggesting that the following whine still influenced phonotaxis. The whine's influence was further confirmed when bats were given the complex calls but with the natural order reversed: chuck-whine vs. chuck (CW vs. C). If only the leading calls were considered, there should have been equal attraction. The bats, however, approached the chuck-whine significantly more often than expected if they had no preference ($t = 3.5$, $df = 9$, $p = 0.007$; Fig 4f).

## DISCUSSION

Frog-eating bats forage in a complex acoustic environment where they are faced with the perceptual problem of locating a single prey item in a chorus. Female frogs are faced with a similar problem as they also use these signals to locate and assess potential mates amidst overlapping calls and heterospecific noise. Given that both of these receivers are localizing the same signal in the same environment we might expect convergence in signal processing. There is evidence for convergence despite substantial differences in peripheral (e.g., cochlea vs. amphibian and basilar papillae) and central (e.g., mammalian cortex in bats) auditory processing. In particular, the auditory system of *Trachops cirrhosus* has neuroanatomical adaptations that extend its frequency sensitivity into the sonic range (Bruns et al. 1989), enabling the detection of frog calls. Túngara frogs also have adaptations in both the peripheral and central auditory systems that enable them to respond preferentially to conspecific calls (Wilczynski et al. 2001; Hoke et al. 2004). Additionally, both taxa can process the two call components separately, allowing for components to be compared between calls. For example, when comparing complex calls with different numbers of chucks, both frogs and bats use the relative difference in call complexity (Akre et al. 2011). Such convergence has influenced the same phonotactic task with different functional outcomes (i.e., a mate verses a prey item, with the latter being a more general or multi-species category). We further tested the extent of convergence by measuring bat responses to call components known to elicit specialized grouping or sorting responses in female frogs.

Our data show that the influence of the two call components on phonotaxis differs between the taxa. When female frogs group call components the whine functions in both recognition and location (so called “what” and “where”) decisions, whereas the chuck only functions in location decisions (Farris et al. 2002; 2005; Farris and Ryan 2011). For bats, however, we found that both components can be used for recognition and location, but components are not equally weighted. First, bats prefer whines over single chucks. With the exception of temporal order, we did not test which acoustic parameter is responsible for this decision. Furthermore, although bats make phonotactic decisions by comparing the relative number of chucks between calls (Akre et al. 2011), our data (e.g., whine-chuck is more attractive than the identical chuck alone) show that this comparison requires whines in both calls. Bats’ greater perceptual weighting of the whine, however, is affected by temporal sequence, as the whine is less attractive when following a chuck. This is in contrast to female frogs, that group single whines and chucks even when presented in non-natural sequences (Farris et al. 2005; Farris and Ryan 2011). A potential reason for bat preferential weighting of the first call component may result from the need to approach a calling frog quickly without alerting the frog to predator presence, as frogs that detect approaching bats cease calling (Tuttle et al. 1982). Bat response is not entirely dictated by component order, however, because the relative preference for the chuck and the whine does not reverse when the order is reversed: some preference for the whine remains. Given the importance of duration to signal detection (e.g. Campbell 1963), the longer duration of the whine compared to the chuck may be a factor affecting bat preference for the whine component.

Our results lead to the conclusion that bats exhibit more flexibility than frogs in call processing, as the chuck alone is sufficient to elicit phonotaxis in bats but not in frogs. The lack of flexibility is not surprising for the frogs, since females are under intense selection to mate preferentially with conspecifics due to the reproductive costs of heterospecifics matings. The bat response matches the flexibility that these bats show in other assessments of prey-generated acoustic cues (Page and Ryan 2005), making it apparent that bats, unlike frogs, do not use the same simple grouping rule as a solution to the problem of assigning call components to a single male in a chorus that is analogous to the acoustic 'cocktail party problem' (Cherry 1953). Indeed, as a generalist predator, assigning call components to a particular source, although beneficial in comparing sources, may limit the ability to locate a source quickly. Speed notwithstanding, bats should not respond randomly to any leading acoustic stimulus. Bats must discriminate palatable from poisonous and smaller from larger anurans by their calls (Tuttle and Ryan 1981). Differences in whine and chuck weighting could therefore be based on strategies best suited for recognizing palatable prey, a selective pressure not shared by female frogs. The response of these generalist bats is likely to be a balance between speed and accuracy, and exhibits little convergence with more specialized female frogs in these complex stimulus binding tasks.

## ACKNOWLEDGEMENTS

We thank the staff of the Smithsonian Tropical Research Institute for assistance with permits and logistics. This research was approved by the Panamanian Authorities

(Autoridad Nacional del Ambiente - ANAM permit # SE/A-91-09; SE/A-95-10; SE/A-6-11; SE/A-46-11; SE/A-94-11) and the Smithsonian Institutional Animal Care and Use Committee (IACUC protocol 20100816-1012-16). Thanks to Victoria Flores, Teia Schwiezer, Jay Falk, Kristina Ottens, May Dixon and Jessica Jacobitz for help capturing and caring for bats. Thanks to Teague O'Mara for statistical advice. PLJ was funded by a National Science Foundation Graduate Research Fellowship and fellowship support from the Smithsonian Tropical Research Institute. HEF was funded by National Institute of Health grant # P20RR016816.

# CHAPTER 2

# Population and seasonal variation in response to prey calls by an eavesdropping bat[2]

## ABSTRACT

The fringe-lipped bat, *Trachops cirrhosus*, is an eavesdropping predator that hunts frogs and katydids by approaching these prey's sexual advertisement calls. In captivity bats can rapidly learn to associate novel acoustic stimuli with food rewards. It is unknown how this learning ability is related to foraging behavior in the wild where prey and the calls that identify them vary over space and time. In two bat populations that differ in available prey species (Soberanía, Panama and La Selva, Costa Rica) we presented wild-caught bats with frog calls, katydid calls, and control stimuli. Bats in Soberanía were significantly more responsive to complex calls and choruses of the túngara frog, *Physalaemus pustulosus,* than were bats at La Selva. La Selva bats were significantly more responsive to calls of the *Steirodon* katydids than bats in Soberanía. We also examined seasonal variation in bat response to prey cues. Bats were captured in Soberanía in dry and wet seasons and presented with the calls of a dry season breeding frog (*Smilisca sila*), a wet season breeding frog (*P. pustulosus*), and four katydid species. Bats captured in the dry season were significantly more responsive to the calls of *S. sila* than bats captured in the wet season, but there was no seasonal difference in response to

---

[2] Published as: Jones PL, Ryan MJ, Page RA (2014*)* Population and seasonal variation in response to prey calls by an eavesdropping bat. *Behavioral Ecology & Sociobiology* 68:605-615. Author Contributions: Jones designed the experiment, conducted the research and wrote the manuscript. Ryan and Page provided advice on experimental design and edited the manuscript.

the calls of *P. pustulosus* or the katydid calls. We demonstrate plasticity in the foraging behavior of this eavesdropping predator, but also show that response to prey cues is not predicted solely by prey availability.

## INTRODUCTION

Learning is thought to be an adaptation for variable environments (Dukas 1998, Shettleworth 1998, Dukas 2004). Learning abilities tested in the laboratory can have positive effects on proxies for fitness. For example, the opportunity to learn associations between sensory cues and nutritive food increases the growth rate of grasshoppers in the laboratory (Dukas and Bernays 2000), and in bumblebees learning capabilities tested in the laboratory predict foraging success in the wild (Raine & Chittka 2008). For many animals, however, it is unknown how learning abilities demonstrated in the laboratory are related to behavior in the wild. We examined whether a bat predator for which learning abilities have been demonstrated in the laboratory exhibits flexibility in response to natural spatial and temporal variation in prey availability.

Learning may be particularly important for predators that locate prey using species-specific prey-generated cues. Eavesdropping predators hunt their prey by approaching the calls prey produce to attract mates (Peake 2005, Zuk and Kolluru 1998). Prey availability to eavesdropping predators is therefore determined by prey signaling behavior as well as prey abundance. The prey that are available to eavesdroppers vary between populations with different prey species as well as within a population if there is seasonal variation in prey calling behavior. This variation in the availability of prey cues might favor the ability of eavesdroppers to learn to recognize the calls of novel prey, and to increase or decrease their responsiveness to prey cues as prey availability changes over time.

The Neotropical fringe-lipped bat, *Trachops cirrhosus*, is an eavesdropping predator on frogs (Tuttle and Ryan 1981) and katydids (Tuttle et al. 1985). In captivity bats are very flexible in their foraging behavior and can rapidly learn to ignore the calls of palatable prey and approach the calls previously associated with poisonous prey (Page and Ryan 2005). After exposure to a trained conspecific tutor, naïve individuals can also learn from experienced bats to approach cues previously associated with poisonous prey (Page and Ryan 2006) or completely novel stimuli such as cell phone ringtones (Jones et al. 2013).

It is unknown how the learning observed in this species in captivity is related to foraging in the wild. We hypothesized that the learning abilities of *T. cirrhosus* enable individuals to flexibly shift their prey preferences with prey availability. The range of *T. cirrhosus* extends from southern Mexico through Brazil (Cramer et al. 2001) and encompasses a variety of frog and insect communities. Bats in the area near the Panamá Canal (Soberanía National Park and Barro Colorado Island) approach speakers broadcasting the calls of a number of frog species (Tuttle and Ryan 1981, Ryan and Tuttle 1983). They also approach the calls of Phaneropterine katydids (Orthoptera: Tettigoniidae), but preferentially approach frog calls over katydid calls (Tuttle et al. 1985).

We examined the responses of wild-caught adult bats to prey calls in two populations that differ in available prey: Soberanía National Park, Panamá and the La

Selva Biological Station, Costa Rica, with the prediction that bat responsiveness would correspond with prey availability. Two frog species with calls to which *T. cirrhosus* in Soberanía are very responsive, the túngara frog, *Physalaemus (=Engystomops) pustulosus* and the pug-nosed treefrog, *Smilisca sila*, are absent from La Selva. We presented bats from both populations with the calls of *P. pustulosus* and *S. sila* to examine how La Selva bats respond to the calls of these allopatric frog species. The eavesdropping behavior of *T. cirrhosus* in La Selva has not been documented, and the diet of bats in this population is unknown. We also presented bats with calls from other frog species that are present in both populations and with katydid calls to determine which prey species the La Selva bats are likely to be eating and if there are population differences in bat response to the calls of prey that are available in both populations.

The absence of the túngara frog, *P. pustulosus*, from La Selva also provided an opportunity to investigate the role of signal complexity in bat preference for prey cues. Male *P. pustulosus* can produce simple calls: a frequency-modulated ~350 ms sweep ("whine") and complex calls: a whine followed by 1-7 broadband ~40–80 ms harmonic bursts ("chucks") (Ryan 1980). Bats, like female frogs, preferentially approach speakers broadcasting complex calls over speakers broadcasting simple calls (Ryan 1980, Tuttle and Ryan 1981). Several hypotheses have been posited for why bats prefer complex calls, including increased localization acuity, higher prey density, and sensory bias. Flight cage experiments show that complex calls are easier for bats to localize under certain conditions (Page and Ryan 2008). Field observations demonstrate that frogs are more likely to make complex calls when there are other frogs calling nearby (Bernal et al.

2007), making complex calls indicative of higher prey densities. A recent field experiment from Ecuador suggests that *T. cirrhosus* could have a sensory bias for complex calls (Trillo et al. 2013). The sister taxa to *P. pustulosus*, *P. petersi,* has populations in Ecuador where frogs only produce simple calls and other populations in which frogs can produce complex calls (Boul et al. 2004). Bats in populations where the frogs do not make complex calls still preferentially approach speakers broadcasting complex *P. petersi* calls (Trillo et al. 2013). We included simple calls, complex calls, and choruses of *P. pustulosus* as stimuli in our population comparison to examine whether bats in La Selva that have never been exposed to *P. pustulosus* calls exhibit a similar preference for call complexity.

We also examined if bat responsiveness shifts over time as prey calling behavior changes seasonally within Soberanía. *Physalaemus pustulosus* breeds and calls primarily in the wet season (ca. May to November); *S. sila,* in contrast, breeds during the dry season (Ibáñez et al. 1999a). We predicted that bats would be more responsive to *P. pustulosus* calls in the wet season, and more responsive to *S. sila* calls during the dry season. Bats in Soberanía also eavesdrop on katydid calls (Tuttle et al. 1985). It is unclear how much seasonal variation there is in katydid abundance in Panamá (Wolda 1978, Richards and Coley, Richards and Windsor 2007). To our knowledge no study has examined seasonal variation in katydid calling behavior, but there is evidence for fluctuations in katydid calling behavior with the lunar cycle (Lang et al. 2006). We included the calls of four katydid species in our stimuli to examine whether there were seasonal differences in bat response to katydid calls. Together these studies provided a unique opportunity to examine how foraging flexibility demonstrated in captivity might function in the wild.

## METHODS

### Study Animals

A total of 43 *T. cirrhosus* were tested in this study. Bats were captured in mist-nets placed at night across streams and trails in the forest. For the population comparison bats were captured at the La Selva Biological Station, Costa Rica (N = 5 bats tested April to May 2010), and in Soberanía National Park, Panamá (N = 5 bats tested June 2010). The seasonal comparison was conducted in Soberanía in two dry seasons (N=4 bats tested January to March 2010, N=10 bats tested January to March 2012) and two wet seasons (N=5 bats, tested June to July 2010 and N=14 bats tested June to November 2011). All bats were adult males or adult non-reproductive females; no bats were used in both experiments or tested in both seasons. For each bat (with the exceptions of one bat from La Selva, and two bats from Soberanía) we divided measurements of capture mass by forearm length to obtain a body condition score (Kelly et al. 2008). For the population comparison bats were immediately released into an outdoor flight cage with ambient light, humidity, and temperature (6m x 4m x 2.5m at La Selva and 5m x 5m x 2m in Soberanía). Bats were tested the night following capture (approximately 24 hours post-capture). In the intervening time bats were provided with 4-6 katydids to forage on within the cage. This was consistent at both sites. For the seasonal comparison bats were maintained in a small (142cm x 127cm x 203cm) mesh tent where they were handfed baitfish for the first 36 hours, and then released into the 5m x 5m x 2m flight cage. Testing began the following evening, approximately 48 hours after capture. At the completion of testing bats were individually marked using Passive Integrated Transponders (PIT tags, Trovan Ltd., U.K.), and released at their capture sites.

### Experimental Protocol

Bats in the population comparison received 11 stimuli (Fig. 5). The first La Selva bat received 5 of the 11 stimuli, and the second La Selva bat received 10, all other bats received the complete set of stimuli. The stimuli consisted of calls of two frog species present only in Soberanía: *P. pustulosus* and *S. sila,* and the calls of four potentially palatable prey species present in both locations: three frog species (*Dendropsophus ebraccatus*, *Dendropsophus phlebodes* and *Smilisca phaeota*), and a Phaneropterine katydid in the genus *Steirodon* (due to difficulties in identification the species is either *S. rufolineatum* or *S. sandrae*). To examine the effect of signal complexity on the responses of naïve bats, we used simple calls, complex calls, and choruses of *P. pustulosus* as separate stimuli. We presented bats with the calls of the poisonous cane toad *Rhinella marina* as a natural control to assess general responsiveness to acoustic stimuli. We also included a 900 Hz tone that tested if the two populations were equally responsive to novel acoustic cues in the sonic frequency range. Our final control was a time-reversed *P. pustulosus* call, thus an upward frequency sweep rather than the normal downward sweep. This stimulus was included because it contains the same frequency and energy content as the natural simple *P. pustulosus* call but sounds quite different to human ears.

Bats in the seasonal comparison were presented with 9 stimuli (Fig. 5): simple calls, complex calls, and choruses of *P. pustulosus*; calls of *S. sila*; calls of the katydids *Balboana tibialis* (Pseudophyllinae), *Ischnomela gracilis* (Pseudophyllinae), *Neoconocephalis affinis* (Copiphorinae), and *Steirodon* sp. (Phaneropterinae); and the 900 Hz tone as a control for seasonal differences in response to acoustic stimuli. Six of the bats in the seasonal comparison had one stimulus for which the video recording was not high enough quality to analyze the bat's response and therefore was excluded from analysis (a dry season bat's *I. gracilis*, and the following recordings from wet season bats: *Steirodon* sp., two tone presentations, a *P. pustulosus* simple call, and a *P. pustulosus* chorus).

Stimulus playback was conducted through one of two speakers placed on the floor in the same corner of the cage connected to a Lenovo ThinkPad T500 laptop. Frog calls and control stimuli were broadcast with a RadioShack FE-103 40-1197 4W RMS/8W MAX speaker through a Realistic SA-150 Integrated Stereo Amplifier. Due to their high frequencies the katydid calls were played with an Avisoft Scanspeak Ultrasound speaker through an Avisoft UltraSoundGate Player 116 (Avisoft Bioacoustics, Berlin, Germany). A wire-mesh screen (1.5m x 1.5m) covered in leaf-litter was placed over the two speakers to conceal them, and each speaker was moved between stimulus presentations, although they always remained approximately 1m apart. Small pieces of baitfish were placed on top of the screen over the speakers as rewards to maintain the bats' motivation throughout the night. Rewards were placed on the speaker broadcasting the stimulus as well as the silent speaker. Bats never landed on the silent speaker, indicating that they were responding to the acoustic stimulus and not cues associated with the reward.

**Stimuli**

Katydid calls were recorded by Hannah ter Hofstede from wild-caught individuals on Barro Colorado Island (see ter Hofstede et al. 2010 for details). Frog calls were recorded by Ximena E. Bernal, Roberto Ibáñez, Patricia L. Jones, Rachel A. Page, A. Stanley Rand, Michael Reichert and Michael J. Ryan. Frog calls were recorded with either a Nagra reel-to-reel tape recorder; a Sony cassette recorder; or a Marantz PMD661 digital recorder and a Sennheiser microphone. All recordings were made in the Soberanía area (Ibáñez et al. 1999b). The number of individuals from which we have recordings for each species is listed in Fig. 5.

The peak amplitude of each stimulus was normalized using Adobe Audition version 3.0. To approximate natural amplitude levels frog calls and other sonic stimuli were broadcast at 70 dB SPL (re. 20 $\mu$Pa) 1m from the speaker and katydid calls at 93 dB SPL 1m from the speaker. We attempted to present all stimuli at their natural duty cycle. The stimuli were broadcast for 10 calls or until the bat landed on the speaker, whichever came first. Three of the stimuli (*P. pustulosus* chorus, the toad *R. marina,* and the katydid *N. affinis*) were continuous and could not be partitioned into discrete calls, therefore they were broadcast for 30 seconds or until the bat landed on the speaker. Each bat received each stimulus only once and the order of presentation was randomized.

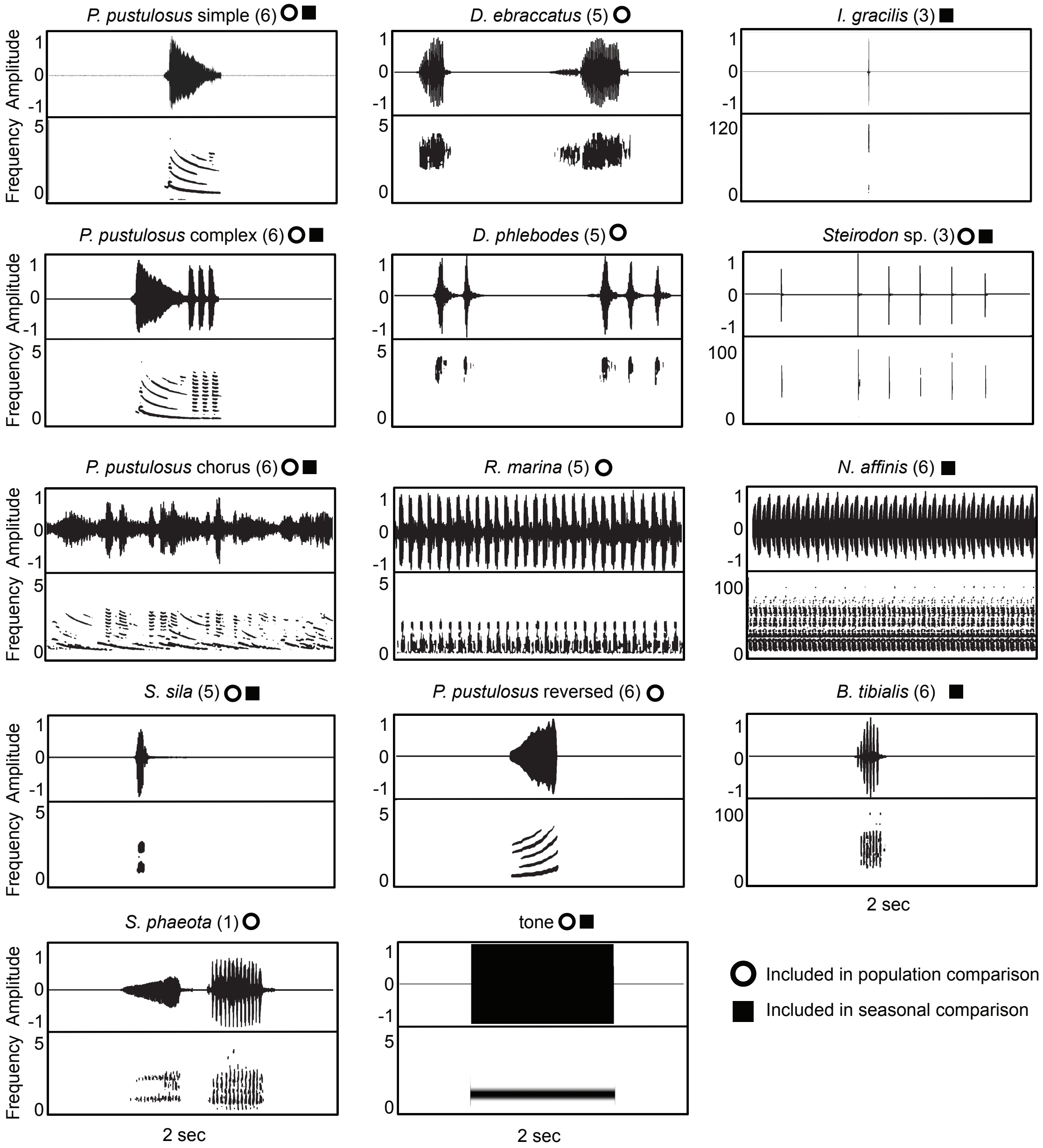

**Figure 5:** Waveforms and spectrograms (in kHz) of the stimuli used in the two experiments.

Spectrogram scales are not the same across all of the panels. The number in parentheses indicates the number of different individuals from which recordings were made

## Response Variables

Audio-video recording of all trials were made for later analysis with a Sony Handycam DCR-SR45 using the "Nightshot" setting and an additional Sony HVL-ILM Battery IR Light (DC7.2V 2W/1.5Vx2). The audio track allowed the viewer to determine onset and offset of stimuli. We recorded three behavioral measures to assess the bats' responses to each stimulus: (1) proportion of the stimulus (time for continuous stimuli or number of calls for discrete stimuli) to which the bat moved its ears, (2) proportion of the stimulus to which the bat's head was oriented to the corner of the cage where the speakers were placed, and (3) whether or not the bat flew towards the speakers. The screen covering the two speakers and the video recording equipment were always placed in opposite corners of the flight cage. These fixed locations allowed us to assess orientation and flight direction when analyzing the video. Each of these behaviors is a different requisite stage in the chain of events between detection of a stimulus and the decision whether to attack. The analysis of each of these behaviors allowed us to catalog different levels of bat responsiveness.

## Statistical Analyses

Analyses were conducted using R version 3.2 (R Core Team 2013). Condition score and forearm length were compared between populations, and condition score between seasons using Wilcoxon rank sum tests. We analyzed each of the three different behavioral responses separately using mixed-effects models with the lme4 package in R (Bates et al. 2013) with individual bat as a random effect. For flight to the speaker we used a mixed model with a binomial distribution. For each stimulus and each behavior we then conducted planned comparisons of between seasons or between populations using the glht function from the R multcomp package (Hothorn et al. 2008).

## RESULTS

### Bat Size and Body Condition

We found no significant difference in bat size (mean forearm length ± SD, median forearm length) between La Selva (58.17 ± 1.10 mm, 58.4 mm, N=4) and Soberanía (57.84 ± 0.71 mm, 57.7 mm, N=5, Wilcoxon rank sum test: $W$=7, $P$= 0.56). There was also no difference in condition score (mean ± SD, median) between the La Selva bats we tested (0.56 ± 0.046g/mm, 0.59g/mm) and the Soberanía bats (0.53 ± 0.025g/mm, 0.53g/mm, Wilcoxon rank sum test: $W$=8, $p$=0.73). Our sample of bats did not provide evidence for an effect of size or body condition on population differences in foraging behavior. We also found no significant difference in condition between bats captured in Soberanía during the dry season (mean ± SD = 0.54 ± 0.051g/mm, N=13) and the wet season (mean ± SD = 0.55 ± 0.047g/mm, N=18, Wilcoxon rank sum test: $W$ = 104, $P$=0.62). Our data provides no evidence that the seasonal differences in bat response to prey cues we observed are products of differences in body condition.

### Population Comparison

Analysis of ear motions in response to the experimental stimuli indicated a marginally significant effect of population ($X^2$ (1, N = 10) = 3.51, $p$ = 0.061), a significant effect of stimulus ($X^2$ (10, N = 10) = 31.71, $p$ < 0.001) and a significant interaction effect ($X^2$ (10, N = 10) = 30.60, $p$ < 0.001) (Fig. 6A). When we analyzed orientation to the speakers we found no significant effect of population ($X^2$ (1, N = 10) = 0.049, $p = 0.83$) or of stimulus ($X^2$ (10, N = 10) = 8.047, $p = 0.62$), but a significant interaction effect ($X^2$ (10, N = 10) = 32.33, $p < 0.001$) (Fig. 6B). The bats we tested from the two populations therefore did not differ statistically in responsiveness, but that there were population differences in bat response to specific stimuli. We also analyzed flight to the speaker and found no significant effect of stimulus ($X^2$ (10, N = 10) = 8.17, $p = 0.61$), or population ($X^2$ (1, N = 10) = 0.00, $p = 1.00$), or interaction effect ($X^2$ (10, N = 10) = 0.00, $p = 1.00$) (Fig. 6C).

From our planned comparisons La Selva bats exhibited significantly more ear motions and orientation to the speakers than Soberanía bats to calls of the katydid *Steirodon* sp. (ear motions: $z = -2.41$, $p = 0.016$, orient: $z = -2.80$, $p = 0.0052$). Two bats from La Selva flew to the speaker in response to the *Steirodon* sp. call whereas no bats from Sobernía approached the stimulus, but this difference was not statistically significant ($z = -0.0020$, $p = 0.99$). La Selva bats also moved their ears and oriented to a significantly higher proportion of the playback of the *D. phlebodes* treefrog than Soberanía bats (ear motions: $z = -2.59$, $p = 0.0095$, orient: $z = -1.969$, $p = 0.049$), and one La Selva bat flew to the speaker ($z = -0.001$, $p = 0.999$). La Selva bats also moved their ears to significantly more of the toxic toad, *R. marina*, call than Soberanía bats ($z = 2.71$, $p = 0.0066$), but there was no significant difference in orientation (z = 1.252  p = 0.211) and no bats in either population flew to the speaker broadcasting the toxic toad calls ($z = 0.00$, $p = 1.00$).

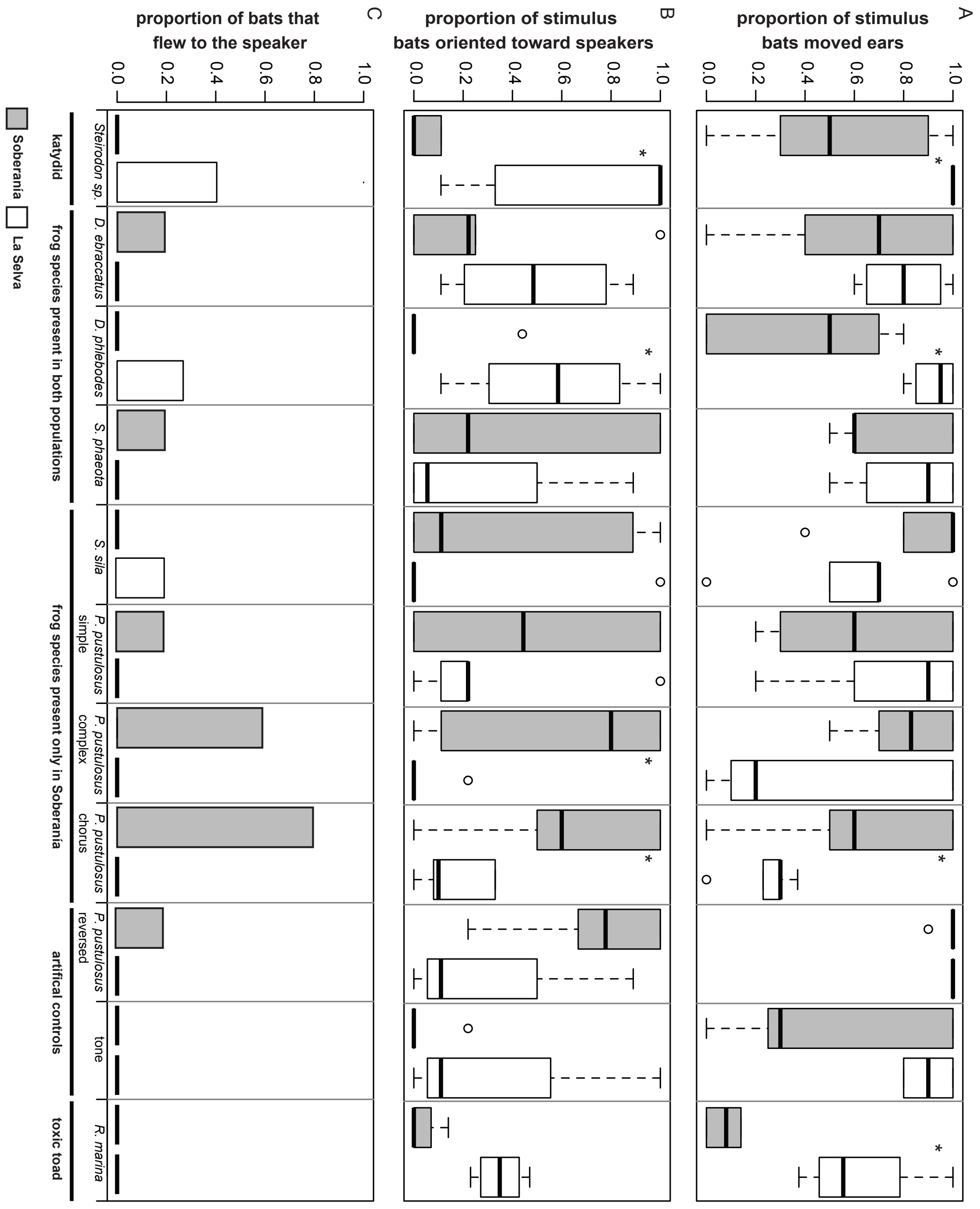

**Figure 6:** Population differences in bat response to each of the experimental stimuli.

**Figure 6:** Population differences in bat response to each of the experimental stimuli. Data from bats captured Soberanía are in gray and bats captured in La Selva are in white. A) Boxplot of the proportion of the stimulus that bats captured in La Selva and Soberanía moved their ears. The bold lines indicate the median and the edges of the boxes indicate the first and third quartiles. Whiskers extend to the lowest datum that is 1.5 times the inter-quartile range from the lower quartile and the highest datum within 1.5 times the inter-quartile range from the upper quartile. Open circles indicate outliers. Asterisks indicate significant differences ($p<0.05$) between the two populations for that stimulus. B) Boxplot of the proportion of the stimulus that bats oriented their heads towards the corner of the cage containing the speakers. C) Barplot of the proportion of bats that flew in the direction of the speaker

Soberanía bats oriented towards a significantly greater proportion of the *P. pustulosus* complex calls and *P. pustulosus* choruses than La Selva bats (complex: $z = 2.34$, $p = 0.019$, chorus: $z = 1.97$ $p = 0.049$). Soberanía bats also moved their ears to a significantly higher proportion of the chorus ($z = 1.99$, $p = 0.046$), and there was a trend but no statistically significant difference in ear motions for the complex call ($z = 1.81$, $p = 0.070$). We also found no significant population difference in flight to the speaker for either stimulus (complex: $z = 0.0020$, $p = 0.99$, chorus: $z = 0.002$, $p = 0.999$), although four Soberanía bats flew to the *P. pustulosus* chorus and three flew to the *P. pustulosus* complex call, whereas no bats from La Selva flew to either stimulus.

We found no significant population differences in the bats' responses to *P. pustulosus* simple calls (ear motions: $z = -0.63$, $p = 0.53$, orient: $z = 0.78$, $p = 0.44$, flight: $z = 0.00$, $p = 1.00$), or calls of *S. sila* (ear motions: $z = 1.36$, $p = 0.17$, orient: $z = 0.87$, $p = 0.39$, flight: $z = -0.001$, $p = 0.999$). Both of these are the calls of prey only present in Soberanía. There were also no significant population differences in bat response to the calls of the treefrogs *S. phaeota* (ear motions: $z = -0.42$, $p = 0.67$, orient: $z = 0.81$, $p = 0.42$, flight: $z = 0.0020$, $p = 0.99$) and *D. ebraccatus* (ear motions: $z = -0.89$, $p = 0.37$, orient: $z = -0.81$, $p = 0.42$, flight: $z = 0.0020$, $p = 0.99$); which are present in both populations. Additionally we found no statistically significant population differences in ear motions or flight in response to the reversed *P. pustulosus* simple call (ear motions: $z = -0.10$, $p = 0.92$, flight: $z = 0.0020$, $p = 0.99$). For orientation to the speaker there was a trend towards Soberanía bats orienting to a higher proportion of the reversed *P.*

*pustulosus* call, but the difference was not statistically significant (orient: $z = 1.88$, $p = 0.060$). There was also a trend towards La Selva bats moving their ears to a larger proportion of the playback of the artificial tone but this was not statistically significant ($z = -1.93$, $p = 0.054$). There was no population difference in orientation or flight to the tone (orient: $z = -1.06$, $p = 0.29$, flight: $z = 0.00$, $p = 1.00$).

**Seasonal Comparison**

In the seasonal comparison we found a significant effect of stimulus on all of the bat behavioral responses (ear motions: $X^2$ (8, N = 33) = 291.13, $p<0.0001$, orient: $X^2$ (8, N = 33) = 237.25, $p<0.0001$, flight: $X^2$ (8, N = 33) = 38.06 $p<0.0001$), but no effect of season (ear motions: $X^2 = 1.03$, $p = 0.31$, orient: $X^2 = 0.098$, $p = 0.75$, flight: $X^2 = 0.12$, $p = 0.73$), or interaction effect (ear motions: $X^2 = 4.74$, $p = 0.79$, orient: $X^2 = 15.50$, $p = 0.05$, flight: $X^2 = 5.00$, $p = 0.76$; Fig 7). The Soberanía bats we tested were therefore significantly more responsive to some stimuli than others, but there was no overall effect of season on their response. Planned comparisons indicated that bats tested during the dry season oriented to a significantly higher proportion to the calls of *S. sila* (dry season calling treefrog species) than bats tested during the wet season ($z = 2.46$, $p = 0.014$), and flew to the stimulus significantly more often ($z = 2.04$, $p = 0.042$). There was no seasonal difference in ear motions for *S. sila* ($z = 1.11$, $p = 0.27$).

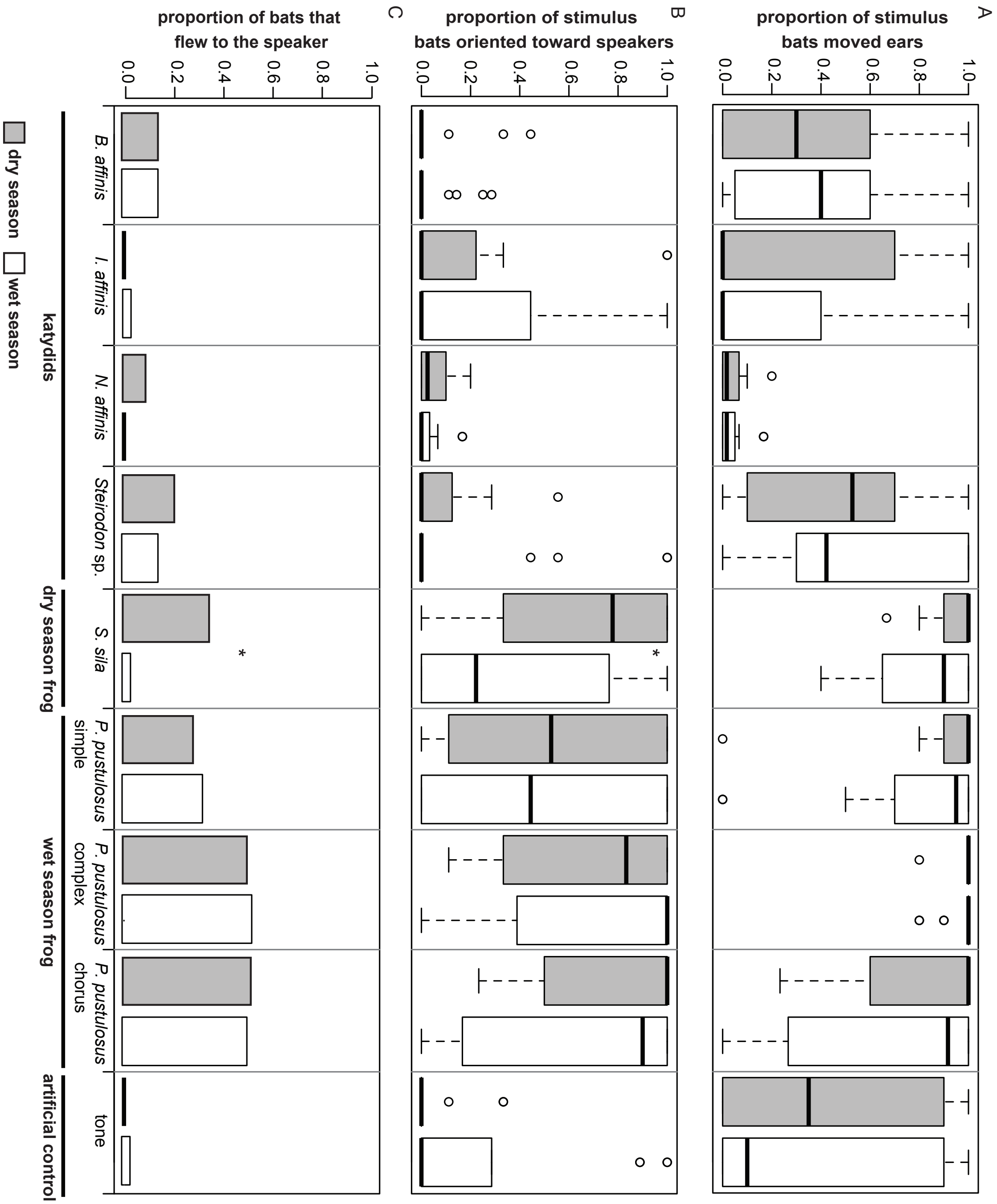

**Figure 7:** Seasonal differences in bat response to each of the experimental stimuli.

**Figure 7:** Seasonal differences in bat response to each of the experimental stimuli. Data from bats captured in the dry season are in gray and bats captured in the wet season are in white. A) Boxplot of the proportion of the stimulus that bats captured in the dry season and the wet season moved their ears. The bold lines indicate the median and the edges of the boxes indicate the first and third quartiles. Whiskers extend to the lowest datum that is 1.5 times the inter-quartile range from the lower quartile and the highest datum within 1.5 times the inter-quartile range from the upper quartile Open circles indicate outliers. Asterisks indicate significant differences ($p<0.05$) between the two seasons for that stimulus. B) Boxplot of the proportion of the stimulus that bats oriented their heads towards the corner of the cage containing the speakers. C) Barplot of the proportion of bats that flew in the direction of the speaker

*Physalaemus pustulosus* predominantly calls during the wet season but we found no significant seasonal difference in bat response to *P. pustulosus* simple calls (ear motions: $z = 1.15, p = 0.25$, orient: $z = 0.20, p = 0.84$, flight: $z = -0.33, p = 0.74$), complex calls (ear motions: $z = -0.16, p = 0.87$, orient: $z = 0.17, p = 0.87$, flight: $z = 0.12, p = 0.90$), or choruses (ear motions: $z = 1.62, p = 0.11$, orient: $z = 1.42, p = 0.16$, flight: $z = 0.1, p = 0.89$). We also found no seasonal differences in bat response to the katydid calls: *B. tibialis* (ear motions: $z = 0.65, p = 0.52$, orient: $z = -0.19, p = 0.85$, flight: $z = 0.090, p = 0.93$), *I. gracilis* (ear motions: $z = -0.51, p = 0.61$, orient: $z = 0.42, p = 0.68$, flight: $z = 0.01, p =1.00$), *Steirodon* sp. (ear motions: $z = -0.32, p = 0.75$, orient: $z = -0.50, p = 0.61$, flight: $z = 0.44, p = 0.66$), *N. affinis* (ear motions: $z = 0.090, p = 0.93$, orient: $z = 0.16, p = 0.87$, flight: $z = 0.010, p = 0.99$), or to the control tone (ear motions: $z = -0.28, p = 0.78$ orient: $z = -1.06$, $p = 0.29$, flight: $z = 0.01, p = 1.00$). Overall, bats captured in Soberanía in the dry season and the wet season responded similarly to all the stimuli presented except the calls of *S. sila*.

## DISCUSSION

Availability of prey to a predator depends on the predator's means of locating and capturing prey (Faure and Barclay 1992). In the case of eavesdropping, prey availability is determined largely by prey signaling behavior. Different predator populations have different prey species with different signals, and within a population there is often seasonal variation in prey signaling behavior. We examined whether the responses of eavesdropping bats to prey sexual advertisement calls differed between populations with different available prey or across seasons within a population. In our population comparison the bats that we tested were different in their response to a number of prey cues. The Soberanía bats exhibited more ear motions and orientation to the speaker in response to complex calls and choruses of the túngara frog, *P. pustulosus,* than the bats from La Selva where *P. pustulosus* is absent. The La Selva bats, in contrast, exhibited more ear motions and orientation to the speaker to calls of the treefrog, *D. phlebodes*, and a Phaneropterine katydid species in the genus *Steirodon*, than bats from Soberanía although these prey are present in both populations. There were also differences in the numbers of bats that flew to the speaker in response to each of these stimuli, but likely due to the small sample sizes, these differences were not statistically significant. Although it is a small sample, these results point toward population specialization in the behavior of this eavesdropping predator.

Population differences in response to prey cues and foraging behavior have been demonstrated for a number of species (e.g. snakes: Arnold 1977, spiders: Hedrick and Riechert 1989, bumblebees: Ings et al. 2009, crayfish: Pintor and Sih 2009). For these species population differences in behavior have a substantial genetic component. Given the ability of *T. cirrhosus* to rapidly learn novel associations between prey cue and prey quality in captivity (Page and Ryan 2005, 2006), it seems more likely that the population specializations we observed in *T. cirrhosus* are learned. Learned population differences in foraging behavior are not as well documented as genetic differences, but there is evidence of such in primates (capuchins: Panger et al. 2002, chimpanzees: Whiten et al. 1999, orangutans: van Schaik et al. 2003). In some of these primates, foraging behaviors are socially learned. *T. cirrhosus* is capable of learning novel prey cues socially (Page and Ryan 2006, Jones et al. 2013), but learned population differences could also arise from individual trial and error learning.

*Trachops cirrhosus* could also learn to prefer calls with complex components. Previous studies have demonstrated that the bats in Soberanía and on nearby Barro Colorado Island preferentially approach complex *P. pustulosus* calls over simple calls (Ryan et al. 1982, Akre et al. 2011). La Selva bats, however, exhibited lower responses (in terms of ear motions and orientation to the speaker) to complex calls and choruses of *P. pustulosus* than to simple calls. When presented with novel stimuli, therefore, the *T. cirrhosus* we tested were not more responsive to more complex stimuli or stimuli that represent multiple individuals calling (choruses). Our result conflicts with research from Ecuador where bats preferentially approach speakers broadcasting complex *P. petersi* calls even in a population where frogs do not make complex calls (Trillo et al. 2013). This discrepancy could be due to a number of factors. Our sample size was small, and the study in Ecuador quantified the number of passes bats made over speakers in the wild, and was not able to determine how many individual bats made such passes. It is also possible that the preference for call complexity in Ecuador may be because bats already have experience with simple calls of that prey species, which could be a pre-requisite for a preference for call complexity. Bats in Soberanía may also have learned their preference for complex calls after having higher capture success approaching complex calls than approaching simple calls. Higher capture success could arise because complex calls are easier to localize (Page & Ryan 2008) or because frogs are more likely to make complex calls when they are at higher densities (Bernal et al. 2007) thereby providing more capture opportunities for a bat approaching a complex call.

If bats do learn to alter their foraging behavior to take advantage of available prey, then we would also expect seasonal variation in response to prey cues as prey availability changes seasonally. In our seasonal comparison we found that Soberanía bats oriented to a significantly higher proportion, and flew significantly more often, to the calls of the dry season breeding frog *S. sila* in the dry season when it was currently calling, but there was no seasonal difference in any bat response to calls of the wet season breeding frog *P. pustulosus*. We therefore demonstrate seasonal variation in bat response to some prey cues but not to others. It is clear from the bat responses in both the population and seasonal experiments that *P. pustulosus* is the preferred prey of all the stimuli offered to Soberanía bats. It is possible that this high preference for *P. pustulosus* results in a consistently high response to calls regardless of temporal availability. *Smilisca sila*, in contrast, is less preferred than *P. pustulosus*. Bats may maintain a high response to preferred prey regardless of availability, but for less preferred prey bat response is affected by availability.

Shifting foraging behavior with prey availability has benefits and risks. While it may enable access to novel food sources, trying novel food is always associated with the possibility that such food could be unpalatable or otherwise dangerous to consume (many katydids have a strong bite). Whether or not to learn novel prey could therefore be viewed from the perspective of risk-prone or risk-averse foraging strategies (Stephens 1981). Risk-prone is defined as a preference for high variability in food resources, whereas risk-averse is a preference for low resource variability (Barnard and Brown 1985). High flexibility in foraging behavior with frequent shifts to novel prey that may or may not produce high rewards could be viewed as risk-prone, whereas low flexibility could be categorized as risk-averse. Whether animals engage in risk-prone or risk-averse foraging behavior can be affected by a number of factors, including body condition and age. Over the course of their lifetimes individual bats may make many decisions about when to engage in risk-prone behavior and learn novel prey and when to be risk-averse and stick with prey that they have prior experience with. Current foraging success on known prey has been shown to affect social learning of novel prey (Jones et al. 2013), but this may be only one of multiple factors that generate foraging flexibility.

There were a number of stimuli for which we found no population or seasonal difference in response. For all of these stimuli bats exhibited a generally low overall response. In our population comparison these included the treefrogs *D. ebraccatus* and *S. phaeota*, and our control stimuli. These treefrogs may not be palatable, may be inaccessible, or may simply be a low-preferred prey. It should also be noted that the call recordings used in this experiment were made in Soberanía. We do not know of geographic variation in the calls of these frog species, but it is possible that La Selva bats would have responded slightly differently if we used local recordings. If this was the case we might expect lower responses in La Selva to non-local individual calls than in Soberanía where the calls were of local individuals, but the La Selva bats we tested were not significantly less responsive to any of these calls. It is therefore unlikely that the low bat response was due to the origins of the recordings.

The low response we found to the artificial control stimuli indicates no population or seasonal differences in response to novel acoustic cues. Bat response to katydid calls was low across both seasons in Soberanía. Bats in La Selva, however, were very responsive to the calls of the *Steirodon* katydid, significantly more responsive in orientation and ear motions than bats in Soberanía. It is possible that the presence of *P. pustulosus* in Soberanía, for which bats in Soberanía exhibit a strong preference, may result in a decreased preference for alternative prey, such as katydids. Similarly, the presence of *P. pustulosus* in Soberanía may reduce response to calls of the treefrog *D. phlebodes* that is present in both populations but to which bats in La Selva exhibited more ear motions and orientation towards the speakers.

In the population comparison we found a significant difference in the proportion of the stimulus to which the bats moved their ears for the toxic toad, *R. marina*. We did not find significant differences for any of the other behavioral measures. This large, unpalatable toad species is abundant at both sites. The significant difference appears to be a product of the very low variance in response by the Soberanía bats. La Selva bats on average did not move their ears to a larger proportion of the toxic toad than to the artificial tone. No bats in either population flew to approach the stimulus. This example highlights the interesting complexity that emerges from examining three different behavioral measures. Each of these behaviors indicates a different step in the range of bat responses between first detecting the stimulus and attack. High levels of ear motions appears to be required for flight to the speaker, but is not necessarily predictive, as indicated by some bats that moved their ears to large proportion of the artificial tone but did not fly to the speaker. Analysis of multiple behaviors gives a more complete picture of bat response.

The foraging behavior of *T. cirrhosus* arises from auditory specializations for detecting frog calls (Bruns et al. 1989) and glandular specializations for consuming frogs (Tandler et al. 1996) most likely in combination with the honing of preferences through learning to associate particular cues with their prey. Our study does not rule out that bat populations have diverged genetically to produce the observed population differences in response to prey cues, and considerable genetic variation in at least one gene (CO1) has been demonstrated for *T. cirrhosus* over its range (Clare et al. 2011). Given the learning abilities demonstrated for *T. cirrhosus*, however, it is likely that learning plays an important role in the development of bats' associations between prey cue and prey quality. The flexibility in response to prey cues demonstrated experimentally for *T. cirrhosus* may enable bats to focus their attention on preferred prey but also take advantage of novel prey as it becomes available. Whether or not individual bats learn novel prey may be affected by a number of factors including body condition and age. The circumstances under which bats undertake the risk of approaching novel prey is in need of further investigation. By demonstrating natural variation in bats' responses to some prey cues but not to others, our results indicate that prey availability is not the only factor affecting bat response to prey cues. This highlights the complexity of the foraging behavior of this eavesdropping predator.

## ACKNOWLEDGEMENTS

We thank the staff of the Smithsonian Tropical Research Institute and the Organization for Tropical Studies for aid and support with research and permits in Soberanía and at La Selva. Thank you to Hannah ter Hofstede for providing the katydid calls, and Ximena E. Bernal, Roberto Ibáñez, A. Stanley Rand and Michael Reichert for recording frog calls. Thanks also to Sara Troxell, Sean Griffin, Martha Moscoso, Christina Buelow, Sarah Richman, Jay Falk, Teia Schweizer and Victoria Flores for help capturing and caring for bats. We thank the Gamboa Bat Lab, Christian Voigt, Brock Fenton, and one anonymous reviewer for insightful comments on the manuscript. This study was funded by a National Science Foundation Graduate Research Fellowship (P.L.J), as well as grants from the University of Texas at Austin (P.L.J.) and the Smithsonian Tropical Research Institute (P.L.J.).

# CHAPTER 3

## When to approach novel prey cues? Social learning strategies in frog-eating bats[3]

### ABSTRACT

Animals can use different sources of information when making decisions. Foraging animals often have access to both self-acquired and socially-acquired information about prey. The fringe-lipped bat, *Trachops cirrhosus*, hunts frogs by approaching the calls that frogs produce to attract mates. We examined how the reliability of self-acquired prey cues affects social learning of novel prey cues. We trained bats to associate an artificial acoustic cue (cell phone ringtone) with food rewards. Bats were assigned to treatments in which the trained cue was either an unreliable indicator of reward (rewarded 50% of the presentations) or a reliable indicator (rewarded 100% of the presentations) and they were exposed to a conspecific tutor foraging on a reliable (rewarded 100%) novel cue or to the novel cue with no tutor. Bats whose trained cue was unreliable and who had a tutor were significantly more likely to preferentially approach the novel cue when compared to bats whose trained cue was reliable, and to bats that had no tutor. Reliability of self-acquired prey cues therefore affects social learning of novel prey cues by frog-eating bats. Examining when animals use social information to learn about novel prey is key to understanding the social transmission of foraging innovations.

---

[3] Published as: Jones PL, Flores V, Ryan MJ, Page RA (2013) When to approach novel prey? Social learning strategies in frog-eating bats. *Proceedings of the Royal Society of London B*. 280:20132330. Author Contributions: Jones designed the experiment, conducted the research and wrote the manuscript. Flores, Ryan, and Page provided advice on experimental design and edited the manuscript.

## INTRODUCTION

Social information, or information acquired from others, is used by a wide variety of taxa in behavioral contexts that range from foraging (e.g. Fisher & Hinde 1949, Galef & Giraldeau 2001, Whiten et al. 2005) to mate choice (e.g. Dugatkin 1992, Dugatkin & Godin 1992, Schlupp et al. 1994, Galef & White 1998). Social learning is an efficient way to acquire information because it avoids costly mistakes that can be made during trial and error learning. Social information, however, can have its own costs in terms of outdated or inaccurate information, or costs associated with interactions with conspecifics (Giraldeau et al. 2002, Laland 2004). These costs and benefits of social information have led to the prediction that animals should use social information selectively in combination with self-acquired information, following particular 'social learning strategies' (Boyd & Richerson 1985, Rogers 1988, Laland 2004, Kendal et al. 2009a). Extensive research in fishes has provided important insights into the role of social information in decisions about where to find food (*reviewed by* Laland 2004, Kendal et al. 2009a, Rendell et al. 2011). Less is known about social learning strategies in animals that use social information to learn novel cues that indicate prey.

Due to the risks of consuming unpalatable food, acquiring information about novel food is suggested as one of the key advantages of social learning (Galef & Giraldeau 2001). Social learning of novel food has been demonstrated for a number of species (e.g. Laland & Plotkin 1991, Sherwin et al. 2002, Thornton 2008). To understand how behavioral innovations might spread through natural populations it is crucial to examine not only whether animals are capable of using social information to learn novel behaviors, but also when animals are likely to use that social information. This has been most thoroughly studied in Norway rats in which naïve observers acquire information about novel food from the breath of experienced conspecifics (Galef & Stein 1985). Satisfaction, uncertainty, predation risk, and environmental stability affect the use of social information in rats (*reviewed by* Galef 2009). This research is key to predicting how and when food preferences are transmitted in groups of rats. Addressing similar questions in non-model systems is important in order to understand the generalizability of social learning strategies. Here we examined social learning strategies in wild-caught frog-eating bats that can use social information to learn novel acoustic prey cues.

The availability of foraging information has been proposed as an advantage leading to the evolution of bat coloniality (Wilkinson 1992), and a number of studies have demonstrated social learning in bats (Gaudet & Fenton 1984, Ratcliffe & ter Hofstede 2005, Page & Ryan 2006, Wright et al. 2011). The fringe-lipped bat, *Trachops cirrhosus*, is a Neotropical carnivore that hunts frog and insect prey by eavesdropping on the prey's mating calls (Tuttle & Ryan 1981, Tuttle et al. 1985). *T. cirrhosus* differentiates poisonous and palatable frog species by their calls (Tuttle & Ryan 1981), but is quite flexible in these associations and can be trained to reverse their preferences (Page & Ryan 2005). Additionally, *T. cirrhosus* can learn novel associations between prey cue and prey quality by observing foraging conspecifics (Page & Ryan 2006). In this study we examined how the reliability of self-acquired cues about prey influences the use of social information to learn novel prey cues.

We created in captivity a scenario in which bats that are foraging on a self-acquired prey cue can interact with a conspecific that is approaching a different, novel, prey cue. We wished to examine how the reliability with which bats received rewards when they approached self-acquired cues affects the use of social information to learn novel prey cues. We used reward schedule (here termed “reliability”) as a proxy for natural capture success. Our prediction was that bats with 50% reliable self-acquired prey cues would be more likely to use social information to learn novel prey cues than bats with 100% reliable self-acquired prey cues. Different prey species emit different acoustic cues, but they may also be associated with particular calling sites. *T. cirrhosus* could learn to approach novel prey by observing a conspecific approach a specific prey cue, or by observing the bat’s approach to a particular location, or by a combination of these mechanisms. We therefore also determined whether bats are more likely to learn to approach the location from which the novel cue was broadcast or the cue itself.

## METHODS

### Experimental animals & arena

We captured bats (N=18 adult males) between February and December 2011 in Soberanía National Park, Panamá. Experiments were conducted in a 5m x 5m x 2.5m flight cage under ambient temperature and humidity, illuminated by a 25W red light bulb. We placed Fostex® FE103En speakers underneath 1.5m x 1.5m screens covered in leaf-litter in two corners of the cage. In the third corner was a shelter to which the bat was trained to return between cue presentations; the experimenter sat in the final corner opposite the shelter with the sound playback and video recording equipment. Sound playback was conducted through a Pyle Pro PTA2 amplifier and a Lenovo T500 Thinkpad laptop. We used two Sony Handycam DCR-SR45 digital video camera recorders and additional Sony HVL-IRM infrared lights to record responses. Before testing, bats that were housed and tested together were given individual-specific haircuts to enable experimenter recognition. After testing, all bats were individually marked with passive integrated transponder (PIT) tags (Trovan Ltd.) and released at their capture sites.

### Experimental overview

The experiment consisted of five components: the initial training phase (at least 40 trials), a set of pre-tests (6 trials), an experimental exposure phase (100 trials), a set of post-tests (6 trials), and a set of cue/location tests (4 trials). A flow chart summarizes the order of the experimental components (Fig. 8); while the subsections below detail the methods used in each individual component.

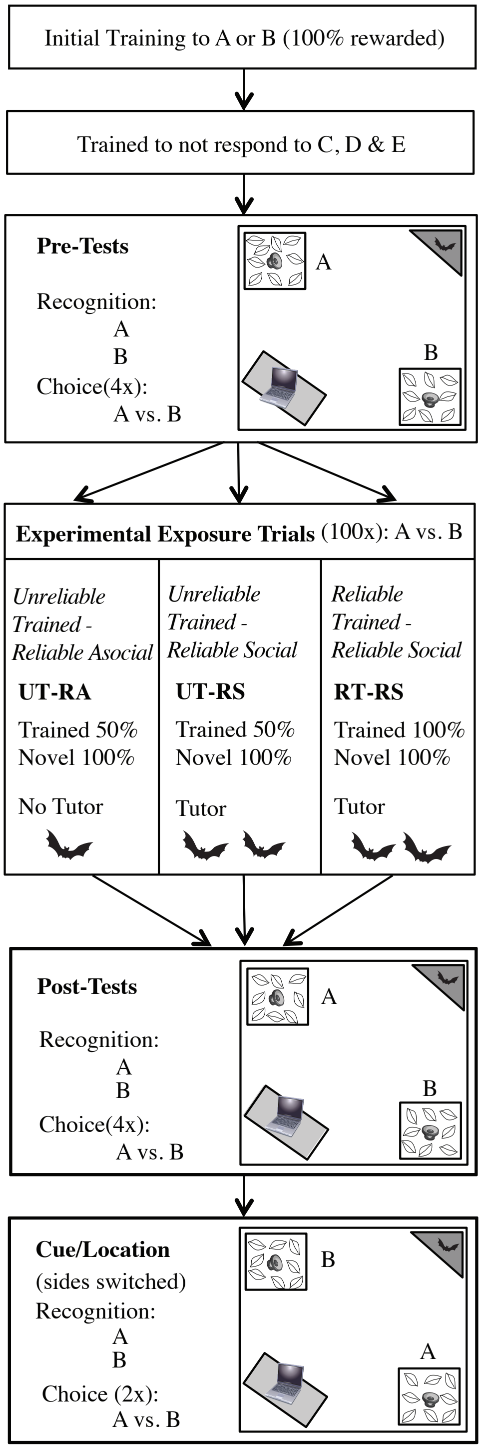

**Figure 8:** Protocol overview. Flight cage diagram is not to scale.

### Initial training

We trained bats to associate one of two cell phone ringtones (A or B, Fig. 9, Supplementary Videos) with food rewards. Ringtones were of approximately the same duration (0.6 sec) and dominant frequency (750 Hz) as the call of the túngara frog, *Physalaemus (=Engystomops) pustulosus*, a preferred prey species of this bat (Tuttle & Ryan 1981), but sounded very different to human ears. We used ringtones to ensure bats had no previous associations with experimental cues. To train bats to approach the ringtones we created stimuli in which we merged the túngara frog call and ringtones using Adobe Audition v. 3.0, and adjusted the relative RMS amplitudes to fade out the frog call and fade in the ringtone in five steps (*see* Page & Ryan 2005). Food rewards consisted of small pieces of baitfish placed on the speaker for each stimulus presentation. Training was completed in one to two nights and the number of trials required (mean trials ± SE: A = 11.3 ± 2.6, B = 13.0 ± 2.1) did not significantly differ between the two cues (Welch two sample t-test: t = -0.5, d.f. = 12.8, p = 0.6). To ensure each bat had ample experience with the trained cue they were presented with the cue associated with a food reward at least 40 times (mean ± SE = 59.3 ± 4.0 trials) before advancing to the pre-tests. During this training period the cue was rewarded every presentation and broadcast alternatively from the two speakers to ensure that bats did not develop a location preference.

Frog-eating bats have been documented to generalize their responses to known stimuli to respond to novel stimuli (Ryan & Tuttle 1983). To ensure that bats were selectively approaching their trained cue and not all acoustic stimuli, we interspersed presentations of the trained cue with presentations of other ringtones (C, D, and E; Fig. 9) that were never associated with food rewards. Generalization was extinguished rapidly with these unrewarded trials (mean number of presentations required for extinction of generalized response ± SE: C = 2.7 ± 0.9, D = 2.3 ± 0.8, E = 3.3 ± 1.4). The number of extinction trials required did not differ significantly between ringtones (negative binomial GLM: $\chi^2 = 0.54$, d.f. = 2, $p = 0.76$). We consistently rewarded the trained cue in these initial training trials to facilitate specific associative learning of the trained stimulus and reduce generalization to the other stimuli (C,D & E).

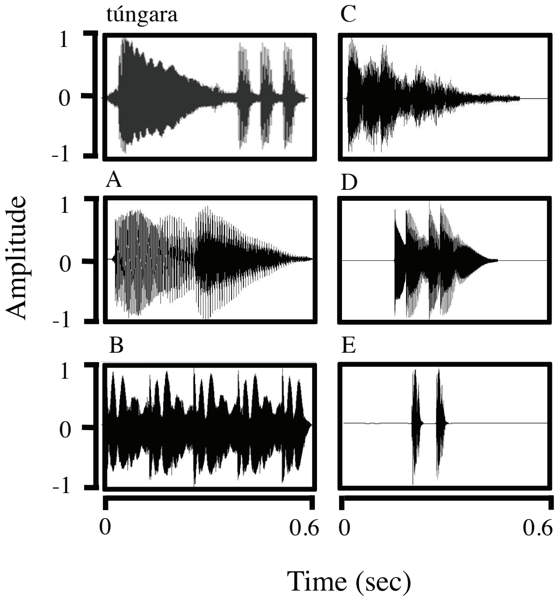

**Figure 9:** Waveforms of experimental stimuli.

The túngara frog call used for training, experimental ringtones/cues A and B, and extinguishing ringtones C, D & E.

### Pre-tests

After training, bats in all treatments were given identical pre-tests to establish a baseline of their responses to the trained cue and the ringtone that would be the novel cue for the experimental exposure trials (A if the trained cue was B or vice versa). Pre-tests were composed of two single-speaker recognition tests (one for A and one for B) and four two-speaker preference tests (A vs. B). Recognition tests determined whether bats responded to the cue; each consisted of one ringtone broadcast from a single speaker 10 times with a 1 s interval of silence between each ringtone, or until the bat landed on the speaker. Preference tests assessed which of the two cues the bat preferred and consisted of presentations of A and B antiphonally from two speakers in opposite corners of the arena (~6 m apart) 10 times or until the bat landed on one of the speakers (*see* Page & Ryan 2005). Pre-tests were rewarded with baitfish placed on both speakers.

### Experimental exposure

For the experimental exposure trials focal bats were randomly assigned to one of three treatments that varied in the reliability (reward schedule) of the trained cue and the presence of a tutor (N=6 bats per treatment). The novel cue was always reliably (100%) associated with food rewards. In the *Unreliable Trained-Reliable Asocial* (UT-RA) treatment the trained cue was 50% rewarded (every other presentation) and the novel cue was broadcast with food rewards placed on the speaker for 100% of the presentations but there was no tutor bat present. In the *Unreliable Trained-Reliable Social* (UT-RS) treatment the cue to which the bats were trained was rewarded 50% of the time (every other presentation) and there was a tutor bat foraging on the reliable novel cue. In the *Reliable Trained-Reliable Social* (RT-RS) treatment the focal bats had a reliable trained cue (maintained 100% rewarded) and they were exposed to a tutor foraging on the reliable novel cue (Fig. 8). Whenever the cue playback was rewarded multiple small food rewards were placed on the speaker. If the focal and tutor bat both approached the stimulus they therefore both had an opportunity to get a food reward, thereby reducing the likelihood of any competition between the two bats.

In the experimental exposure trials stimuli A and B were broadcast antiphonally from the two speakers 10 times each or until one of the bats landed on a speaker. One or both of the two bats (focal bat and tutor) had to approach a speaker in order to proceed to the next trial. The 100 experimental exposure trials required four to five nights to complete. During this time the tutor and focal bat were housed together. We recorded which cue the focal bat approached for each trial. We compared the number of focal bats in each treatment that approached the novel cue during the experimental exposure trials with Fisher's exact tests in R version 2.15 (R Core Team 2012). To examine the effect of treatment on the number of trials required for focal bats to approach the novel cue we conducted negative binomial generalized estimating equations (GLM) using the R MASS package (Venebles & Ripley 2002).

### Post-tests

After the 100 experimental exposure trials the tutor was removed (if present) and the focal bat was given post-tests that were identical to the pre-tests (four preference tests and two recognition tests). We compared the effect of treatment on preference for the novel cue (clustered by individual bat) with a binomial generalized estimating equation (GEE) using the R geepack package (Højsrgaad et al. 2006). We also examined the effect of treatment on the number of bats that approached each cue in the recognition tests using Fisher's exact tests.

### Cue/location tests

The sides of the arena from which the trained and novel cues were broadcast were maintained consistent for the pre-tests, experimental exposure trials, and post-tests. This was a precaution to ensure that the tutor bat consistently approached the novel cue and did not approach the focal bat's trained cue. Focal bats therefore had the opportunity to learn to approach either the novel cue itself or the location from which the novel cue was broadcast. After post-tests bats were given cue/location tests to determine whether bats learned to approach the novel cue or the side of the arena from which the novel cue had been broadcast. Cue/location tests consisted of two preference tests and two recognition tests with the speaker locations opposite those in the post-tests. Due to a logistical problem, one bat in the UT-RA treatment and two bats in the UT-RS treatment did not receive cue/location tests. Within each treatment we compared the proportion of trials bats preferentially approached the novel cue between the post-tests and the cue/location tests using paired t-tests. We then determined if the preference for the cue differed significantly between playback locations. For the recognition tests we compared the number of bats in each treatment that approached the novel cue in the opposite location using a Fisher's exact test. We also examined if bats differed in their recognition of the novel cue depending on whether they had recognized the novel cue in the post-tests using a Kruskal-Wallis one-way analysis of variance.

## RESULTS

### Pre-tests

Focal bats always approached the trained cue and did not approach the novel cue in any of the pre-tests.

### Experimental exposure trials

A few focal bats in each treatment approached the novel cue during the experimental exposure trials. More bats in the UT-RS treatment approached the novel cue than in the other treatments but there was no statistically significant difference (Fisher's exact test: $p = 0.095$). Bats in the UT-RS treatment also approached the novel cue for more of the 100 experimental exposure trials (25.8 ± 10.1 trials) than bats in the RT-RS (2.2 ± 1.1 trials) or UT-RA treatments (11.0 ± 8.5 trials), but the response was not significantly predicted by treatment (negative binomial GLM: $\chi^2 = 4.74$, d.f. = 2, $p = 0.093$, Fig. 10).

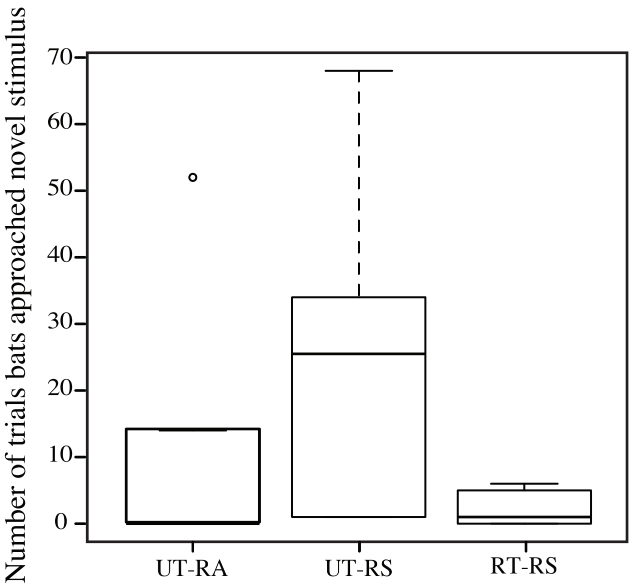

**Figure 10:** Boxplot of the number of experimental exposure trials (out of 100) for which focal bats in each treatment approached the novel cue over the trained cue.

**Post-tests**

We found a significant effect of treatment on preference for the novel cue (GEE: Wald = 319, d.f. = 2, $p < 0.001$, Fig. 11a). When the novel cue had been demonstrated by a tutor bat focal individuals whose trained cue was unreliable (UT-RS) approached the novel cue significantly more than individuals whose trained cue was reliable (RT-RS) (Wald = 8.73, $p = 0.0031$). When the trained cue was unreliable, bats that had a tutor (UT-RS) were significantly more likely to prefer the novel cue than bats that had no tutor (UT-RA) (Wald = 3829, $p < 0.001$). There was no significant difference between the RT-RS and UT-RA treatments (Wald = 0, $p = 1.0$). Therefore reliability of the trained cue and the presence of a tutor in combination affected preference for the novel cue.

We found no significant differences between treatments in recognition of the novel cue (Fisher's exact test: $p = 1.0$, Fig. 4b). A few bats in all of the treatments recognized the novel cue in the post-tests. Most of these bats had approached the novel cue during the experimental exposure trials. Neither the absence of a tutor or a reliable trained cue deterred some bats from investigating the novel cue. There was also no significant difference between treatments in recognition of the trained cue (Fisher's exact test: $p = 1.0$, Fig. 11c). Bats that shifted their preference to the novel cue therefore maintained recognition of the trained cue.

**Cue/location tests**

To determine whether bats had learned the cue or the location from which the cue was broadcast, we reversed the playback locations for the cue/location tests. We found no significant difference in preference for the novel cue between locations (paired t-tests; *UT-RA* $t=-1$, d.f.=4, $p=0.37$, *RT-RS* $t=-0.69$, d.f.=5, $p = 0.52$, *UT-RS* $t=1.57$, d.f.=3, $p=0.23$, Fig. 11a), indicating preference for the cue itself rather than the playback location.

In the cue/location recognition trials, the only bats to approach the novel cue in its new location were those that had a tutor present in the experimental exposure trials, a treatment difference that approached statistical significance (Fisher's exact test: $p = 0.07$, Fig. 11b). Bats that recognized the novel cue in the post-tests approached the novel cue when it was broadcast from a different location significantly more often than bats that had not recognized the novel cue in post-tests (Kruskal-Wallis: $\chi 2 = 8.2$, d.f.=1, $p=0.004$). All bats approached the trained cue when it was broadcast from the new location (Fig. 11c), further demonstrating bat response to the acoustic cue rather than the playback location.

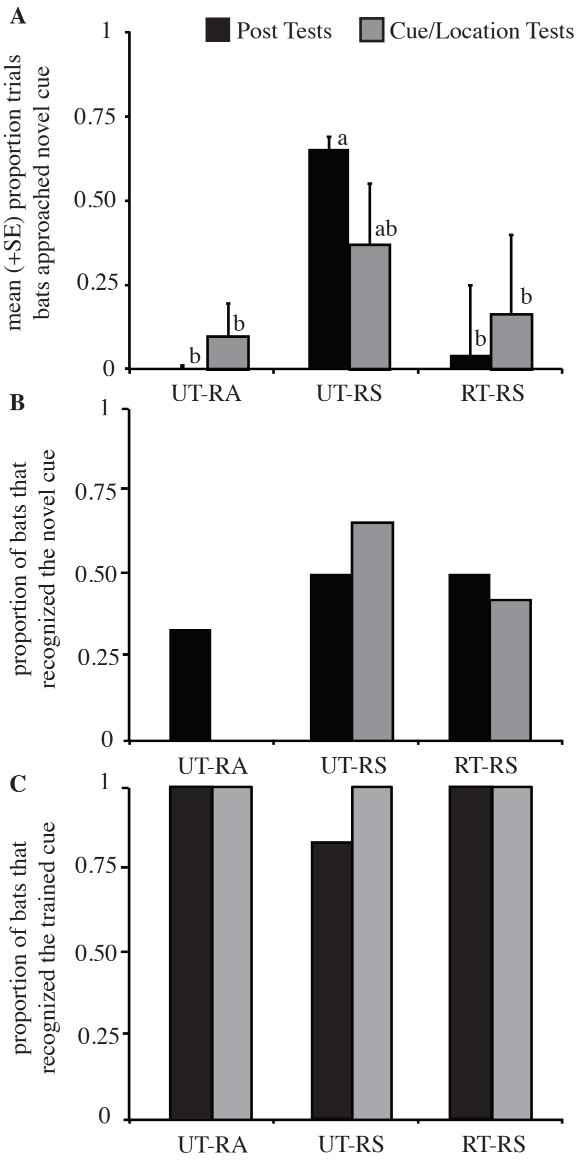

**Figure 11:** Post-test and cue/location test results.

**Figure 11:** Post-test and cue/location test results for the Unreliable Trained – Reliable Asocial (UT-RA), Unreliable Trained – Reliable Social (UT-RS), and Reliable Trained – Reliable Social (RT-RS), treatments. A) The mean and standard error of the proportion of trials for which focal bats approached the novel cue in two-speaker preference tests. Different letters indicate significant differences between groups. B) The proportion of focal bats that approached the novel cue in single-speaker recognition tests. C) The proportion of focal bats that approached the trained cue in single-speaker recognition tests.

## DISCUSSION

We found that bats whose self-acquired prey cues were unreliably associated with rewards were significantly more likely to approach a novel cue demonstrated by a conspecific tutor than either bats with reliable trained cues and a tutor, or bats with unreliable prey cues and no tutor. The reliability of self-acquired prey cues therefore affects the bat's use of social information to learn novel prey cues. This result is consistent with multiple theoretical social learning strategies: 'copy when uncertain', 'copy if better', 'copy when dissatisfied' and 'copy when asocial learning is costly' (Laland 2004, Kendal et al. 2009a). The 50% reward schedule of the self-acquired prey cue could be interpreted as generating uncertainty about prey quality or environmental stability. Previous studies that have examined 'copy when uncertain', however, use uncertainty to refer to when the animal has very little, or conflicting, self-acquired information (Boyd & Richerson 1988, Visalberghi & Fragaszy 1995, van Bergen et al. 2004, Galef et al. 2008, Grüter et al. 2011). The bats in our experiment had substantial experience with their trained cue, and no conflicting information. We therefore feel that 'copy when uncertain', as it has been previously applied, is not likely to be the strategy exhibited by bats in this experiment.

'Copy if better' (Schlagg 1998, Kendal et al. 2009, Pike et al. 2010) is another relevant social learning strategy because the novel prey cue was always 100% rewarded, and thus 'better' than the 50% rewarded self-acquired prey cues in the unreliable treatments. We did not manipulate the reward schedule of the novel prey cue, and therefore did not directly test whether this is the strategy employed by the bats. 'Copy if better' is a relatively sophisticated social learning strategy because it requires animals to evaluate and compare their own success to the demonstrator's success. A much simpler strategy is that individuals copy the behavior of conspecifics when they are dissatisfied or when there are costs to individual learning (Galef et al. 2008, Kendal et al. 2009a, Grüter & Ratnieks 2011). 'Copy when dissatisfied' and 'copy when asocial learning is costly' are consistent with our experiment and do not require animals to assess the demonstrator's success. In *T. cirrhosus*, as in many animals, responding to a prey cue does not reliably result in a meal (Tuttle et al. 1982, Page & Ryan 2008), making approaching prey cues costly and potentially resulting in dissatisfaction. 'Copy when asocial learning is costly' and 'copy when dissatisfied' are likely to be applicable in other taxa that use social information to learn novel foraging behaviors.

A few bats in all of our treatments approached the novel cue in the recognition tests. The availability of reliable known prey and the absence of a tutor do not appear to preclude investigation of novel prey. This disposition towards exploration of acoustic stimuli even in the absence of a conspecific tutor has been demonstrated previously for *T. cirrhosus* (Page & Ryan 2005, Page & Ryan 2006). Only bats that had been exposed to a conspecific tutor, however, preferentially approached the novel cue over the trained cue when the tutor was removed. The presence of a tutor therefore appears to facilitate or reinforce a general tendency towards exploration of novel acoustic cues.

We found considerable individual variation in bat responses to novel prey cues and use of social information. For example, not all bats learned to approach novel cues, and of those that did, most learned the cue regardless of location but a few were affected by playback location. All of the bats in this experiment were wild-captured adults and likely varied in their previous social and foraging experiences. *T. cirrhosus* generally roost together in small groups (Kalko et al. 1999), and it is not unusual to capture two adult males in the same net in close succession (P. Jones *personal observation*), indicating the potential for transfer of foraging information in the wild. The variation we observed in this experiment may result in part from the previous social and foraging experiences of individuals in the wild. One of the advantages of conducting learning experiments with wild-caught adult animals is that it is more likely to encompass behaviorally relevant variation due to previous experience. Results indicating social learning are therefore more robust and ecologically relevant, while at the same time revealing variation that is present in a wild population.

There is a growing literature on the factors that affect the use of social information when animals are presented with conflicting private (self-acquired) and public (social) information (e.g. van Bergen et al. 2004, Duffy et al. 2009, Kendal et al. 2009b, Pike & Laland 2010, Webster & Laland 2011). Many animals, however, may not encounter such conflicts, but rather use social information to expand their behavioral repertoires. The study of when to use social information to learn novel prey or novel behaviors is crucial for understanding how novel behaviors can spread through populations and thereby create the potential for animal culture.

## ACKNOWLEDGEMENTS

We would like to thank the staff of the Smithsonian Tropical Research Institute for their help with logistics and permits. Capture in Soberanía National Park was approved by the Panamanian authorities (Autoridad Nacional del Ambiente - ANAM permit # SE/A-91-09; SE/A-95-10; SE/A-6-11; SE/A-46-11; SE/A-94-11). Animal care was conducted according to approved Institute for Animal Care and Use Committee protocols from the University of Texas (protocol AUP-2009-00138), and the Smithsonian Tropical Research Institute (protocol 20100816-1012-16). Thanks to Christina Buelow, Jay Falk, Sarah Richman, Teague O'Mara, and Teia Schweizer and for help capturing and caring for bats. Stuart Dennis and Teague O'Mara provided statistical advice. John Ratcliffe and Kevin Laland provided helpful comments on the manuscript. This research was funded by an NSF GRFP (PLJ) and grants from the Smithsonian Tropical Research Institute (PLJ), American Society of Mammalogists (PLJ), and Animal Behavior Society (PLJ).

# CHAPTER 4

## Behavioral evidence for eavesdropping on prey song in two Palearctic sibling bat species[4]

### ABSTRACT

Eavesdropping on prey communication signals has never before been reported for a Palearctic bat species. In this study, we investigated whether lesser and greater mouse-eared bats, *Myotis blythii oxygnathus* and *Myotis myotis*, find tettigoniid bushcrickets (Tettigoniidae) by eavesdropping on their mate-attraction song. Tettigoniids are known to be the most important prey item for *M. blythii oxygnathus*, while carabid beetles and other epigaeic arthropods are the most important prey for its sibling species, *M. myotis*, in many places in Europe. *M. myotis* locates walking beetles by listening for their rustling sounds. We compared these two species' response to four acoustic prey cues: calling song of two tettigoniid species, the rustling sound made by walking carabid beetles, and a control tone. Individuals of both bat species attacked the speaker playing tettigoniid song, which clearly indicates that both species eavesdrop on prey-generated advertisement signals. There were, however, species differences in response. *M. blythii oxygnathus* exhibited stronger predatory responses to the calling song of two species of tettigoniid than to the beetle rustling sound or the control. *M. myotis*, in contrast, exhibited stronger predatory responses to the beetle rustling and to one tettigoniid species but not the other

---

[4] Published as: Jones PL, Page RP, Hartbauer M, Siemers BM (2011) Behavioral evidence for eavesdropping on prey song in two Palearctic sibling bat species. *Behavioral Ecology & Sociobiology* 65:333–340. Author Contributions: Jones designed the experiment, conducted the research and wrote the manuscript. Hartbauer, Page, and Siemers provided advice on experimental design and edited the manuscript.

tettigoniid or the control. Our study (1) for the first time demonstrates eavesdropping on prey communication signals for Palearctic bats and (2) gives preliminary evidence for sensory niche partitioning between these two sympatric sibling bat species.

## INTRODUCTION

Eavesdropping on advertisement signals by predators and parasitoids is a behavior that can have important ecological and evolutionary effects. Peake (2005) defines eavesdropping as "the use of information in signals by individuals other than the primary target". Using heterospecific signals to locate prey has been found across sensory modalities, including auditory (Ryan et al. 1982), chemical (Stowe et al. 1995; Roberts et al. 2001) and visual signals (Lloyd and Wing 1983). Eavesdropping can be an important selective force on the mate-attraction signals of prey (reviewed in Zuk and Kolluru 1998), and may also influence the evolution of auditory capabilities (Bruns et al. 1989; Robert et al. 1992), ecology (Tuttle et al. 1985), and foraging behavior (Page and Ryan 2005) of predators. Eavesdropping on prey cues has been described for multiple species of bats in the Southern Hemisphere (Tuttle and Ryan 1981; Tuttle et al. 1985; Belwood and Morris 1987; Ryan and Tuttle 1987; Bailey and Haythornthwaite 1998), and North America (Buchler and Childs 1981; Spangler 1984; ter Hofstede et al. 2008), but never before in the Palearctic region.

Studies of eavesdropping by bats have often examined predation on tettigoniids (Orthoptera: Tettigoniidae) (Spangler 1984; Belwood and Morris 1987; Tuttle et al. 1985; Bailey and Haythornthwaite 1998; ter Hofstede et al. 2008), likely due to the fact that the song of tettigoniids extends well into the ultrasonic range (Heller 1988), exceeding 100 kHz in at least one tropical species (Morris et al. 1994). Therefore, frequency components of many bushcricket songs overlap with the hearing range of microchiropteran bat species, which is typically 20 kHz to 80 kHz (Pollak et al. 1972). In Europe, tettigoniids make up a large proportion of the diet of the lesser mouse-eared bat, *Myotis blythii oxygnathus* (Arlettaz et al. 1997), but it is unknown how *M. b. oxygnathus* locate their prey in the dense grass habitats where they forage (Arlettaz 1999). In this study we investigated whether *M. b. oxygnathus* find tettigoniids by eavesdropping on their calling song.

*M. b. oxygnathus* has a morphologically very similar, but ecologically distinct sibling species, the greater mouse-eared bat, *Myotis myotis* (taxonomy and phylogeography discussed in Ruedi and Mayer 2001 and Bogdanowicz et al. 2009). Studies from central Europe have indicated that *M. myotis* and *M. b. oxygnathus* avoid competition by foraging in different habitats, and by foraging on the different prey associated with those habitats. *M. myotis* predominantly forages in forests and other areas with open, accessible soil (Arlettaz 1999), and mainly preys on large, epigaeic arthropods such as carabid beetles (Arlettaz 1996; Arlettaz et al. 1997), which it finds by listening for their rustling sounds (Kolb 1961; Arlettaz et al. 2001; Siemers and Güttinger 2006). *M. b. oxygnathus*, in contrast, tends to forage in dense grass (Arlettaz 1999) and largely consumes tettigoniids (Arlettaz 1996; Arlettaz et al. 1997). The difference in diet between these two species in central Europe is disparate enough that fecal sample analysis has been suggested as a method for species identification (Arlettaz et al. 1997)

Simulated echolocation calls of *M. myotis* induce *Tettigonia viridissima* to exhibit escape responses (Schulze and Schul 2001), and repetitive ultrasonic 30 kHz pulses (10 ms pulse duration) frequently induce song cessation in this tettigoniid species (M. Hartbauer unpublished). The echolocation calls of *M. b. oxygnathus* and *M. myotis* are very similar (Russo et al. 2007), and this is most likely a generalized response to ultrasonic stimuli. *T. viridissima*'s song cessation in response to ultrasound could indicate that European bats are eavesdropping on tettigoniid song. We investigated whether the two bat species utilize the same prey cues (Tettigonia songs and beetle rustling), or instead if differences in sensory ecology play a role in partitioning their diet spectra.

The means by which sympatric species partition resources to enable stable coexistence have long been a topic of discussion and debate (e.g., Pianka 1981; Hubbell 2001; Tilman 2004). Many studies have examined coexistence maintained by morphological differences between species (reviewed in Schluter 2000). More recently, researchers have begun to examine the role of sensory ecology in partitioning resources between sympatric species (Tuttle et al. 1985; Bernays and Wcislo 1994; Swift and Racey 2002; Siemers and Schnitzler 2004; Siemers and Swift 2006; Safi and Siemers 2010). Differences in sensory ecology could allow one species to take advantage of a resource less accessible to the competing species. For example, a number of bat species detect prey by listening for the sound of prey movement (Fiedler 1979; Anderson and Racey 1991; Faure and Barclay 1992; Swift and Racey 2002; Siemers and Swift 2006). Relying on listening for prey sound versus using high-resolution echolocation allows two morphologically alike bat species to access different types of prey, resulting in dietary niche separation (Siemers and Swift 2006). Bat foraging behavior that utilizes eavesdropping could also have implications for resource partitioning between closely related species (Tuttle et al. 1985).

In this study we tested the following two hypotheses: first we hypothesized that *M. b. oxygnathus* eavesdrop on the mate-attraction signals of tettigoniids to locate individual prey ('eavesdropping hypothesis'). We thus predicted that they would attack or inspect a speaker playing tettigoniid song. Secondly, we hypothesized that the different food niches of the sibling bat species are mirrored in their different responses to acoustic prey cues ('sensory niche partitioning hypothesis'). We predicted that *M. b. oxygnathus* would respond more strongly to playback of tettigoniid song than to playback of the rustling sounds of carabid beetles, and conversely that *M. myotis* would be more attracted to carabid rustling sound than to the song of tettigoniids.

## MATERIALS AND METHODS

### Animals and flight room

Our study was conducted in August 2009 at the Tabachka Bat Research Station (Ruse district, northern Bulgaria); the field station of the MPIO Sensory Ecology Group. Capture, husbandry and behavioral studies were carried out under license of the responsible Bulgarian authorities (MOEW-Sofia and RIOSV-Ruse, 57/18.04.2006, 100/04.07.2007, 193/01.04.2009, and 205/29.05.2009). We adhered to the ABS/ASAB guidelines for ethical treatment of animals. Adult male *M. b. oxygnathus* and *M. myotis* were caught at their roost caves in harp traps before dawn. At the field station, bats were kept under relatively constant, naturalistic temperature regime (14 hours light: 10 hours dark) with ad lib access to water and mealworms. If necessary there were also hand-fed mealworms to maintain capture weight.

Bats were released together into a large flight room (8 m by 4 m; 2 m height) at least 3 hours before dusk to become habituated to the room. At dusk when experiments were initiated, all of the bats in the flight room were caught, and then only one bat was released into the flight room and tested at a time. Upon release the bat was allowed to acclimate for 5 min before the tests began.

### Experimental procedure

Each bat was exposed to a total of four different stimuli; calling song of two different European tettigoniid species (prey mating sounds), the rustling sounds of walking carabid beetles (prey locomotion sounds), and a tone (control). Each of the stimuli was broadcast using Avisoft Bioacoustics Recorder USGH through an Avisoft ScanSpeak Ultrasound speaker (frequency response ±4.5 dB between 5 and 90 kHz) and Avisoft UltraSoundGate Player 116 USB box. The speaker was placed in the center of the room and surrounded by branches with leaves for partial concealment. Four video cameras were positioned so that the speaker was clearly visible, and the video screen was marked so that we could determine when the bat flew within 1 m of the speaker.

Each playback lasted for a total of 6 min; stimuli were broadcast for 30 s and then 30 s of silence followed. We expected bats to approach stimuli quickly, i.e., within the 30 s playback phases. Swift approach is important for efficient foraging and the prevention of prey escape (Nyberg 1971). Approaches during the 30 s silence phases, were regarded to be less likely driven by foraging motivation. We thus compared the number of approaches during playback and silent phases  as a measure of the bats interest in the acoustic stimulus.

Each bat received the four 6-min stimuli in random order with 1 min in between each trial; a total of 27 min of testing for each bat. In the interval between trials we entered the room and if the bat was hanging on the wall gently nudged it into flight to ensure that the bat was equally alert for all tests. During the tests we quantified the number of times the bat approached within 1 m of the speaker broadcasting the stimulus, and recorded whether the approach was in the 30 s when the stimulus was playing or during the 30 s of silence. We also recorded landings on the speaker.

We tested a total of 13 *M. myotis* individuals and 22 *M. b. oxygnathus* individuals. Of the 33 bats tested, 10 (77%) of the *M. myotis* and 13 (60%) of the *M. b. oxygnathus* flew within 1 m of the speaker in response to at least one of the four stimuli. We classified the bats that approached at least one stimulus as "responsive" and excluded the non-responsive bats from our analysis. All bats were released at the site of capture upon completion of the experiments.

### Playback stimuli

Examples of the playback stimuli are depicted in Fig. 12 in spectrogram and waveform representation. We used calling songs from two large European species of tettigoniid: *Tettigonia viridissima* and *T. cantans*. Tettigoniid calling songs were recorded using a ¼” microphone (Type 40BE, G.R.A.S. Inc., frequency range: 10 Hz-100 kHz) at

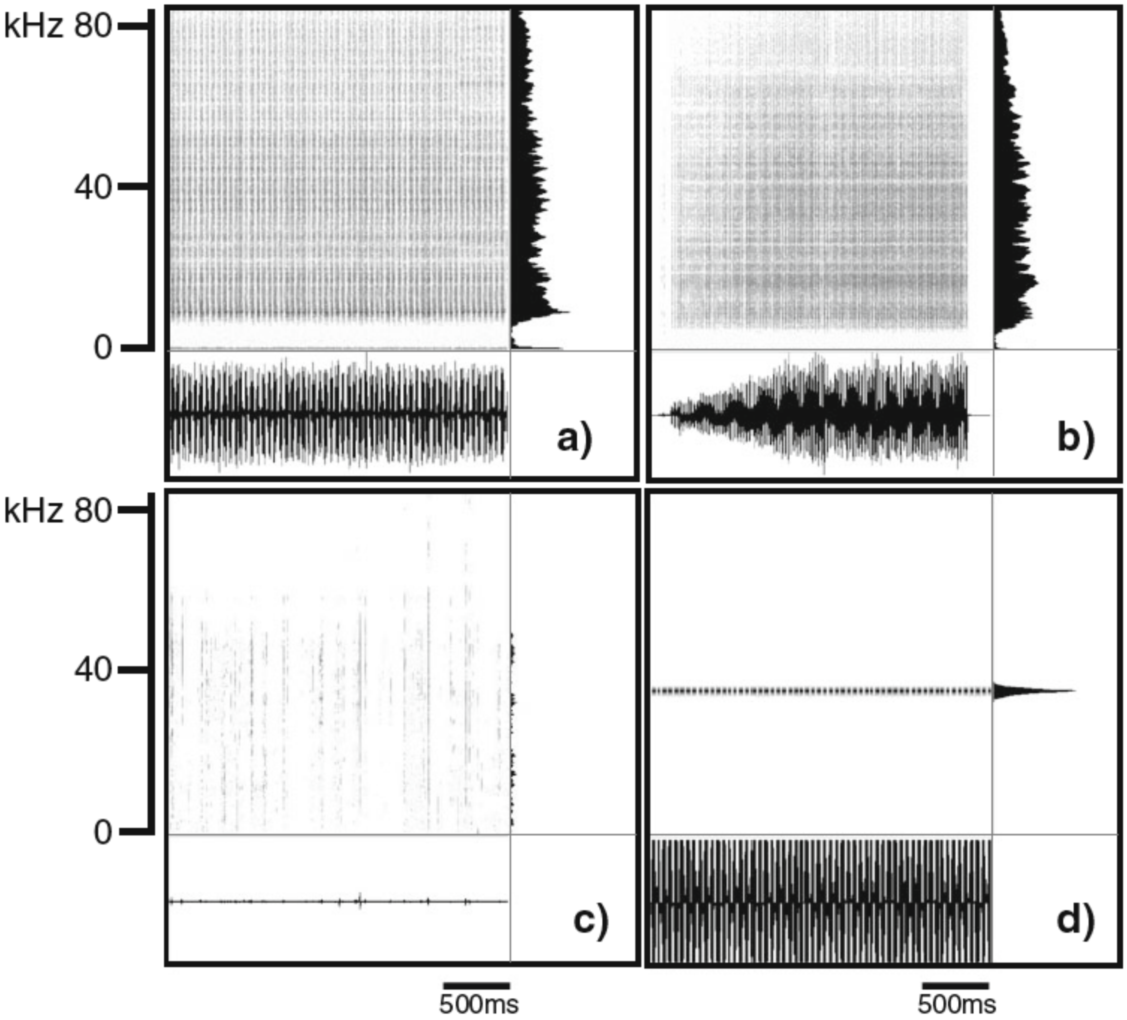

**Figure 12:** Representative examples of the playback stimuli in spectrogram representation with waveform below and averaged power spectrum on the right.

a) Continuous pulsed calling song of *T. viridissima* at 28°C ambient temperature. b) One verse of *T. cantans* song (28°C); verses were repeated with 2-s intervals. c) Rustling sound produced by a carabid beetle (*C. monilis*) walking on dry grass. Note that the amplitude of the rustling sounds is much fainter than the tettigoniid song. d) A pulsed tone as negative control stimulus (35 kHz; pulse duration 30 ms) a distance of 30 cm from a singing male (sampling rate: 166 kHz). For each species we used two different song recordings, made at the University of Graz by one of us (MH) with captive tettigoniids. One *T. viridissima* individual was recorded at 28°C (62.5 Hz syllable rate) and one at 22°C (40 Hz). Similarly, one *T. cantans* was recorded at 28°C (45.5 Hz) and one at 21°C (26.3 Hz). The temperature difference did not affect the bats' behavior and thus for each species, the data from the two temperatures were pooled [*T. cantans*: Fisher's Exact Test (N = 16 at 28°C, 7 at 21°C) p = 0.405, *T. viridissima*: Fisher's Exact Test (N = 17 at 28°C, 6 at 22°C) p = 0.640]. Natural amplitudes for both tettigoniid calling songs are quite loud with peak levels at 1 m of 94 dB SPL for *T. viridissima* (Keuper et al. 1988), and 84-87 dB SPL for *T. cantans* (Hartbauer, unpublished). Tettigoniid songs of both species were average RMS adjusted, and broadcast at an average RMS amplitude of 75 dB SPL, and a peak amplitude of 95 dB SPL, 1 m from the speaker. Playback amplitudes were determined with a broadband Avisoft condenser microphone (Type CM16/CMPA,) and ultrasound recording interface (UltraSoundGate 416H, Avisoft-Bioacoustics, Berlin, Germany) which we had calibrated against a 1/8" measurement microphone (Type 40 DP, G.R.A.S., Holte, Denmark). *T. viridissima* generally sings nonstop for hours, so for this species the 30 s of playback was constant. *T. cantans,* on the other hand, generally sings for 1 - 4 s (mean ± SD: 4.3 ± 1.4 s (21°C); 2.68 ± 0.62 s (28°C)) interrupted by pauses of 3 - 5 s (mean ± SD: 5.7 ± 7.4 s (21°C); 3.78 ± 3.392 s (28°C)) (M. Hartbauer, unpublished). We approximately replicated this natural call timing by playing a 2.4 s call followed by 2 s of silence repeatedly for 30 s.

The third stimulus was a recording of the rustling noises produced when different individual carabid beetles (*Carabus monilis*) walk on dry grass (mown meadow), broadcast at an average RMS amplitude of 46 dB SPL 1 m from the speaker. The rustling sounds were recorded with a ½” high-sensitivity condenser microphone (Type 40HH, G.R.A.S., Holte, Denmark), sampled at 192 kHz and high-pass filtered at 500 Hz; for details see Goerlitz et al. (2008).

The final stimulus was a negative control to which we did not expect the bats to respond. Here, we used Cool Edit 2000 (Syntrillium Inc.) to create a pulsed simulated tone at the same dominant frequency as the *T. cantans* song (35 kHz). Each pulse of sound was 30 ms long and tapered for 5 ms on either side with 15 ms of silence in between. This is approximately the same timing as the sound pulses within the natural tettigoniid song, and was played at a peak amplitude of 96.5 dB SPL 1 m from the speaker.

**Statistics**

Statistics were computed using SPSS 15.0 for Windows. To account for multiple tests per species, we followed Neuhauser (2004) and, in addition to the individual p-values, computed summary p values from the truncated product method (TPM). TPM p-values were calculated using a program (tpm.exe) provided at http://statgen.ncsu.edu/zaykin/tpm/ (see Zaykin et al. 2002) to test whether any of the tests with $p<0.05$ are indeed significant (Neuhauser 2004).

## RESULTS

### Attacks on the speaker

Seven out of the 23 responsive bats briefly landed on the loudspeaker during playback trials (see supplementary videos for examples). Brief landings of this type are typically seen in prey capture bouts in both *M. myotis* and *M. b. oxygnathus* (Arlettaz et al. 2001; Russo et al. 2007; Schaub et al. 2008). A total of 28 landing events occurred, 27 during the 30-s playback phases and a single one during an interspersed 30 s silent phase (Table 1). Each bat showed landing behaviour for just one of the four playback stimuli it received. In all seven bats, this was always an acoustic prey cue and never the control tone. Out of the 28 landings, 27 occurred during playback trials with tettigoniid calling song (six out of seven bats). The single landing attack of the seventh bat was during playback of carabid rustling sound. Individuals of both bat species attacked the speaker during tettigoniid song playback.

| species | individual | *T. viridissima* | *T. cantans* | Rustle | Tone |
|---|---|---|---|---|---|
| *M. myotis* | 39 | 0 | 1 | 0 | 0 |
| *M. myotis* | 65 | 0 | 4 | 0 | 0 |
| *M.b.oxygnathus* | 22 | 0 | 0 | 1 | 0 |
| *M.b.oxygnathus* | 24 | 1 | 0 | 0 | 0 |
| *M.b.oxygnathus* | 14 | 3* | 0 | 0 | 0 |
| *M.b.oxygnathus* | 25 | 0 | 12 | 0 | 0 |
| *M.b.oxygnathus* | 31 | 0 | 6 | 0 | 0 |
| # of individual bats that landed | | 2 | 4 | 1 | 0 |

* One landing event of this bat was during an interspersed 30 s silent phase. All others were during the 30 s playback phases, i.e., swift responses.

Table 1: Number of landing events on speaker for all 7 bats that showed landing behaviour

## Approaches to the speaker

*M. b. oxygnathus* approached the speaker more often during the 30 s playback phases than during the 30 s silent phases for song playback of both tettigoniid species, but not for the rustling of carabid beetles or the control tone (Fig. 13A).

Generally, *M. myotis* was more responsive than *M. b. oxygnathus*, (repeated measures ANOVA, between subjects effect, $F_{1,21}$=7.6, p = 0.012; compare Figs. 13 A and B). Specifically, *M. myotis* approached the speaker more often during the 30 s playback phases than during the 30 s silent phases for the carabid rustling sounds and the *T. cantans* song. There was no difference for *T. viridissima* song or the control tone (Fig. 13B).

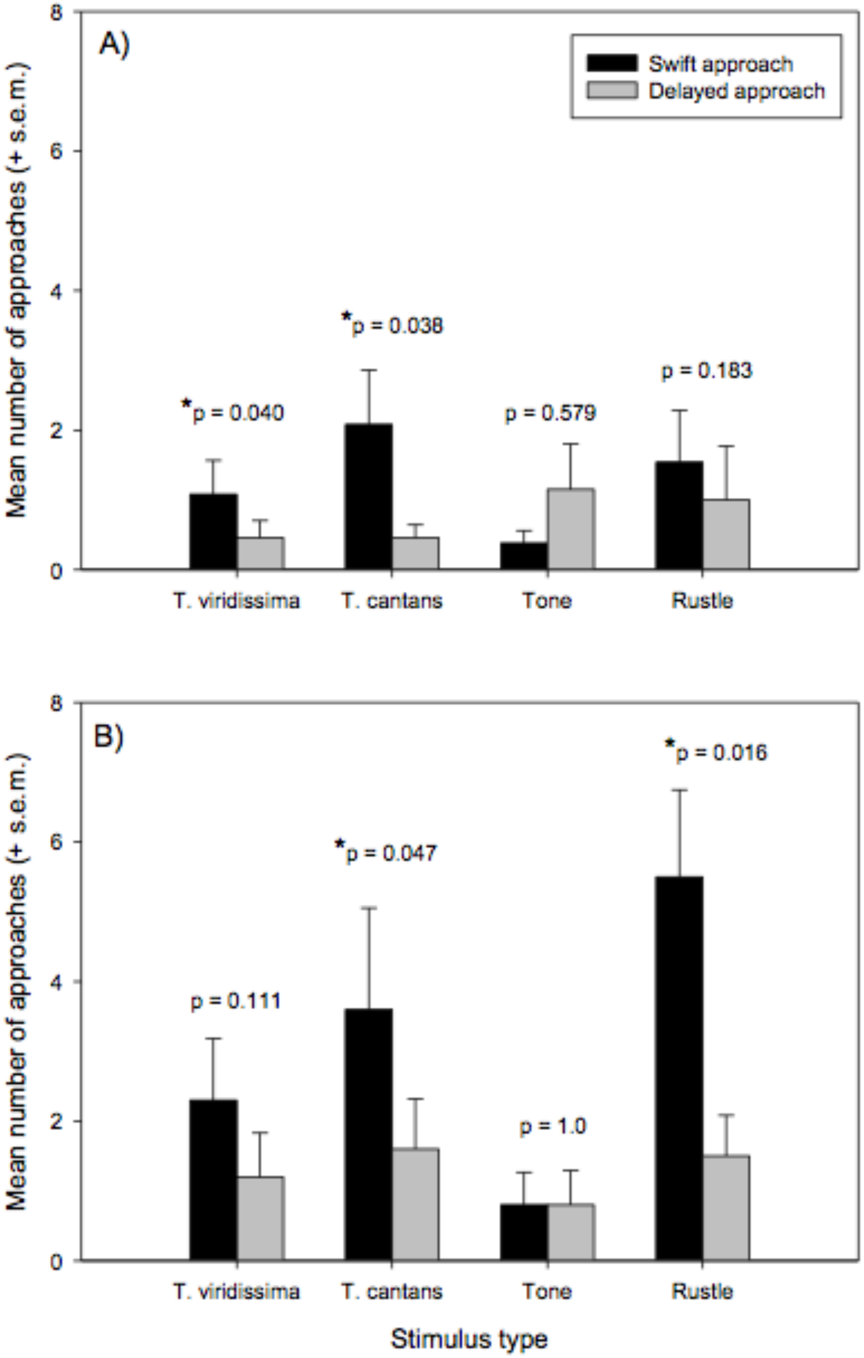

**Figure 13:** Approaches to the speaker for *M. b. oxygnathus* and *M. myotis*.

**Figure 13:** Approaches to the speaker for *M. b. oxygnathus* and *M. myotis*. Mean number of approaches within 1 m of the speaker for each stimulus during the swift approach (playback phase) and the delayed approach (silent phase) for *M. b. oxygnathus* (panel A, n = 13 individuals) and for *M. myotis* (panel B, n = 10 individuals). P-values from paired t-tests are given above each stimulus type. To account for multiple tests per species, we computed summary p values from the truncated product method (see methods). These were p = 0.0111 for *M. b. oxygnathus* and p = 0.0045 for *M. myotis*.

This pattern is also demonstrated in the proportions of individual bats that approached the speaker in response to different stimuli (Fig. 14). A considerably larger proportion of *M. myotis* reacted to the carabid rustling sounds than did *M. b. oxygnathus* (8 out of 10 *M. myotis* versus 5 out of 13 *M. b. oxygnathus*; Chi$^2$ = 4.0, $p = 0.046$).

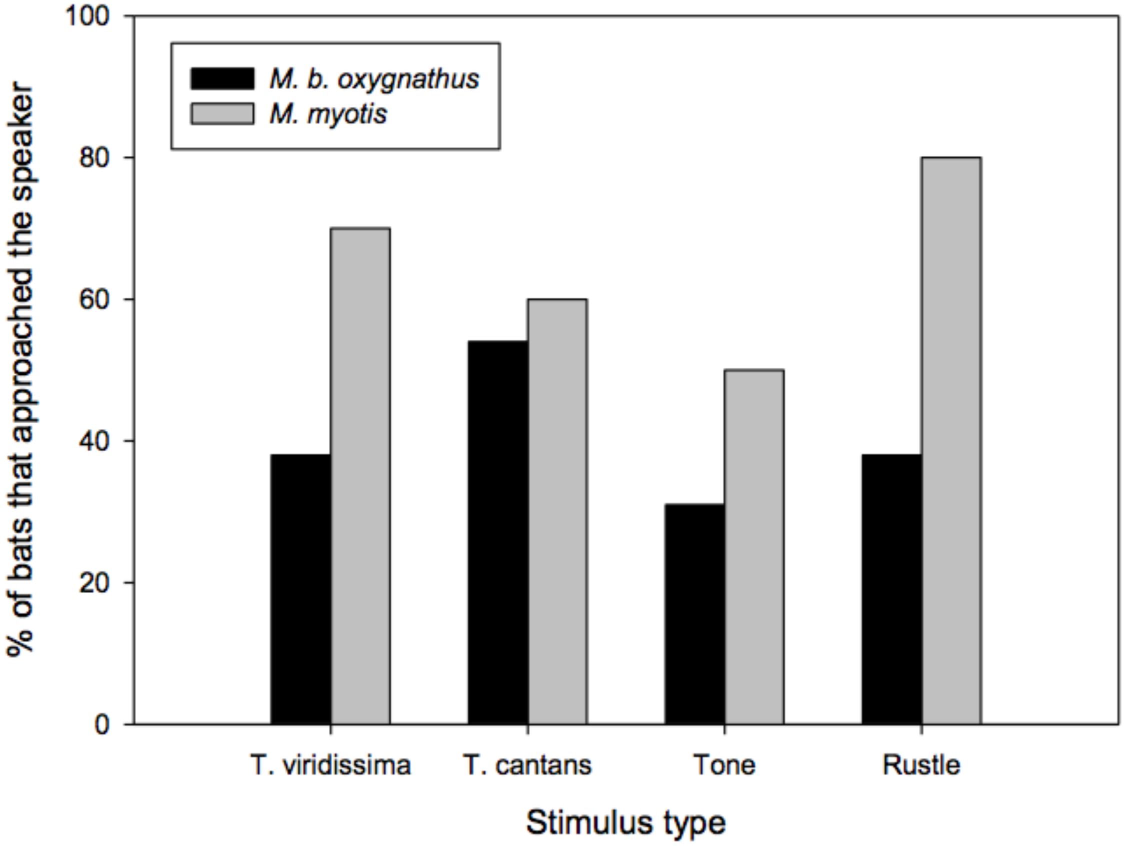

**Figure 14:** Percentage of the responsive bats (individuals that responded to at least one of the stimuli) that approached within 1 m of the speaker.

Percent that approached in the 6 min they were exposed to each stimulus. N, i.e. 100%, was 13 for *M. b. oxygnathus* and 10 for *M. myotis*.

## DISCUSSION

### Eavesdropping on prey mate-attraction signals

Both *M. myotis* and *M. b. oxygnathus* exhibited predatory behavior (landed on the speaker) in response to the song of *T. cantans* and *T. viridissima*, two tettigoniid species that are abundant in Bulgaria (Fauna Europaea). No bats landed on the speaker in response to the control tone, indicating that the response is not generalized to any sound at this frequency but rather particularly to these insects' mate attraction signals. Our experiments thus supported the eavesdropping hypothesis not only for *M. b. oxygnathus*, but also, unexpectedly, for *M. myotis*. This is the first time eavesdropping behavior has been demonstrated in a Palearctic bat species.

Foraging behavior in which bats predate on tettigoniids by eavesdropping on their mate attraction signals could be an important selective force influencing the acoustic communication of tettigoniids. Changes in calling behavior that reduce predation have been demonstrated in temperate (Spangler 1984, Faure and Hoy 2000) and Neotropical tettigoniids (Belwood and Morris 1987). Belwood and Morris (1987) showed that tettigoniid species calling in forested areas with an abundance of foliage gleaning bats (FGB) have lower call duty cycles than species in open areas with fewer FGBs, and species in forested areas used more vibratory communication signals, a behavior which is safe from acoustic eavesdropping and absent in tettigoniid species from open areas. They also found that FGBs are more successful at locating tettigoniid individuals with higher duty cycle calls, indicating that eavesdropping by bats has likely selected for reduced song duty cycle and increased substrate-born signaling in the forest tettigoniid species with which FGBs most frequently co-occur.

Eavesdropping responses of *M. myotis* and *M. b. oxygnathus* to the song of *T. cantans* and *T. viridissima* could therefore be affecting the calling and mate choice behavior of these and other Palearctic tettigoniid species. Male *T. viridissima* experience less call attenuation when they call from higher sites on vegetation, but they are not typically found on the highest vegetation points available (Arak and Eiriksson 1992). This may reflect a trade-off between maximizing the range over which their signals can be detected, and minimizing predation risk, possibly from eavesdropping bats as well as visually hunting birds. For instance, male field crickets, *Teleogryllus oceanicus*, are less likely to be consumed by bats when calling from their preferred refuges than when calling in the open, and they produce longer calls, which are more attractive to bats, from refuges than when in the open (Bailey and Haythornthwaite 1998).

The singing location preferences of the two tettigoniid species, however, are different. *T. cantans* generally sings from relatively conspicuous places on vegetation 0.5-2 m off the ground (Hartbauer, personal observations), while *T. viridissima* males usually sing from more elevated and hidden locations in tall bushes and trees (Arak and Eiriksson 1992; Hartbauer, unpublished data). This difference in conspicuousness of singing sites could render *T. cantans* generally more accessible to bats than *T. viridissima* (Hartbauer, personal communication).

## Sensory niche partitioning

Documentation from Switzerland and Portugal (Arlettaz et al. 1997) demonstrate that tettigoniids are the main prey for *M. b. oxygnathus* in summer and autumn (average May – September percentage volume in fecal samples from Switzerland 60%, with up to 92% in September; from Portugal, 99% in June), and are much less used by *M. myotis* (May –September average below 1% in Switzerland; 13.5% in Portugal in June). It is not entirely appropriate to compare *Myotis* diets from these locations due to differences in climate and potential availability of prey over time, but the differences between species remain striking. Yet, in our study in Bulgaria both *M. b. oxygnathus* and *M. myotis* exhibited clear predatory responses to tettigoniid song. It is possible that there are regional differences in diet between Bulgarian populations and well-studied populations in central Europe and the Iberian Peninsula (Arlettaz 1996; Arlettaz et al. 1997; Pereira et al. 2002, Zahn et al. 2007), such that Bulgarian *M. myotis* are consuming larger proportions of tettigoniids than elsewhere.

Stable isotope analyses of wing tissue indeed indicated that Bulgarian *M. myotis*, in addition to carabid beetles and other secondary consumer arthropods (Bayesian mixing model estimate: 38%), eat up to 62% tettigoniids or other primary consumers, though *M. b. oxygnathus* still consumes 30% more (up to 93%; BM Siemers, S Greif, I Borissov, SL Heucke-Voigt, CC Voigt, unpublished data). Since this data comes from wing tissue rather than fecal samples, the isotope balance is an average over a few months (CC Voigt, personal communication), the rate of wing tissue turnover. Regardless, Bulgarian *Myotis myotis* are consuming a much larger percentage of primary consumers than their central European counterparts. It is remarkable that trophic resource partitioning between these two sibling species is much more clear-cut in Switzerland than in Bulgaria. It would thus be interesting future research to investigate whether the clearer trophic segregation in the Swiss populations is also mirrored in sensory ecology, i.e., in stronger differences in responsiveness to prey stimuli (tettigoniid song versus rustling sounds). Pereira et al. (2002) found that crickets were the preferred prey of *M. myotis* in Southern Portugal, and postulated that this could be due to the crickets' conspicuous calling behavior. If *M. myotis* in Portugal indeed eavesdrop on the calls of crickets when foraging, it is not so surprising that Bulgarian *M. myotis* respond to the calling song of tettigoniids.

Our results support the sensory niche partitioning hypothesis by demonstrating differences in sensory ecology between *M. myotis* and *M. b. oxygnathus* in the predicted direction – *M. myotis* responded more strongly to the rustling sounds of walking arthropods, and *M. b. oxygnathus* more to tettigoniid song - however, these differences were slight. Thus, they are likely not the main factor determining diet partitioning between these two sibling species in the Balkanic area of sympatry. We assume that habitat selection - as documented for Swiss sympatric populations (Arlettaz 1999) - is an additional important mechanism driving resource partitioning.

## Conclusions

The documentation of eavesdropping in a Palearctic bat species, and particularly in two species whose ecology has been relatively well-studied, opens up new areas in both chiropteran and tettigoniid behavioral ecology. From the bat perspective, further investigation is needed to understand the use of eavesdropping as a foraging strategy, e.g. quantifying detection distances for singing tettigoniids and the potential for assessing prey taxon (and thereby profitability or accessibility) from tettigoniid song. In turn, the singing and courtship behavior of Palearctic tettigoniids will need reassessment in view of the predation pressure from eavesdropping bats, and the evolution of appropriate defensive measures.

## ACKNOWLEDGEMENTS

We are grateful to Ivailo Borissov, David Bethel, Tess Driessens, Stefan Greif, Antonia Hubancheva, Markus Schuller, and Sara Troxell for help and company during fieldwork, bat husbandry, and experiments. We thank Maike Schuchmann for assistance in amplitude measurements, and Victoria Grossauer for the evaluation of tettigoniid song parameters. We thank the responsible Bulgarian authorities ((MOEW-Sofia and RIOSV-Ruse) for granting us permission to conduct this research and the Directorate of the Rusenski Lom Nature Park (Director Milko Belberov) as well as the Bulgarian Bat Research and Protection Group for cooperation and support. We are grateful to two anonymous reviewers for their insightful comments on the manuscript. This study was funded by the Max Planck Society (BMS), an Alexander von Humboldt Foundation Postdoctoral Research Fellowship (RAP), and a National Science Foundation Graduate Research Fellowship (PLJ).

# SUMMARY AND DISCUSSION

In the preceding chapters I have investigated some of the factors that influence response to prey cues by the Neotropical fringe-lipped or frog-eating bat, *Trachops cirrhosus*, and the potential for niche partitioning by eavesdropping between two Palearctic sister taxa, *Myotis myotis* and *Myotis blythii oxygnathus*. In the first chapter I examined how *T. cirrhosus* perceives the different components of prey calls. I showed that the way bats weight and group the components of the complex calls of male frogs is very different from how female frogs weight and group the same signal components (Farris et al. 2002). Although these two receivers have converged in phonotaxis to the male signal, their divergent neurophysiogical and cognitive pathways result in different perception of signal components. In this system bats must be able to locate a single prey item to attack amongst a chorus of calling frogs. How bats perceive and group signal components affects which prey they select to attack. This study emphasizes the role of cognition in localization of prey cues by eavesdroppers.

In the second chapter I examined how prey availability affects response to prey cues in *T. cirrhosus*. Eavesdropping predators locate prey using species-specific prey cues (Zuk and Kolluru 1998), which may make successful foraging particularly vulnerable to variation in prey availability. *Trachops cirrhosus* can very quickly learn novel prey cues and learn to reverse their prey cue/quality associations (Page and Ryan 2005). I was interested in whether this flexibility might enable bats to shift their responses to prey cues in accordance with prey availability. I found that bats exhibited population and seasonal differences in response to some prey cues but not to others, indicating that prey availability is likely only one of the factors influencing bat response to prey cues. I continued to investigate foraging flexibility in the third chapter, which focused on when bats learn novel prey cues. In particular I was interested in the conditions under which bats are likely to use social information to learn novel prey cues. I found that bats are more likely to use social information to learn novel prey cues when the prey cue they are currently foraging on is inconsistently associated with rewards. This study is one of the few examinations of the strategies employed by animals for deciding when to learn novel prey cues.

In the fourth and final chapter I examined the eavesdropping behavior of two closely related bat species, the European greater and lesser mouse-eared bats, *Myotis myotis* and *Myotis blythii oxygnathus*. I found that both of these bats respond to katydid calls, the first demonstration of eavesdropping in a Palearctic bat, and there were species differences in how bats respond to katydid calls versus the rustling sounds of beetles moving through leaf-litter. *Myotis myotis* was more responsive to rustling sounds and *M. b. oxygnathus* was more responsive to katydid calls. I therefore demonstrated the potential for niche partitioning through eavesdropping by these morphologically similar sister taxa.

The first three chapters highlight the importance of cognition in foraging behavior. I examined how bats perceive and group components of frog calls to locate individuals, how flexibility in response to prey cues may enable bats to take advantage of available prey, and when bats are likely to learn novel prey cues. Locating prey by eavesdropping requires the predator to differentiate palatable and unpalatable prey by their calls. Many eavesdroppers, including eavesdropping bats, are specialists on one species (Zuk et al. 1993) or group of prey (Cade 1975). *Trachops cirrhosus* is unusual in the variety of prey calls it responds to. In birds there is evidence that generalist predators have better learning abilities than specialists (Greenberg 1983), and the same may be true for bats, and I would argue especially for eavesdropping bats. *Trachops cirrhosus* is a generalist on many different prey types, and has already been demonstrated to learn prey cues quickly (Page and Ryan 2005; Page and Ryan 2006). By focusing on how cognitive abilities influence foraging decisions, my thesis elucidates why bats select to attack particular prey, which in turn can generate natural selection on prey calls and calling behavior.

Eavesdropping has long been studied as a natural selective force on signaling prey (Zuk and Kolluru 1998). For example, in areas where eavesdropping bats are present, katydids call with reduced duty cycles and supplement their calls with vibrational signals that may be harder for bats to detect (Belwood and Morris 1987). Eavesdroppers in turn have adaptations to enable prey localization. For instance, *T. cirrhosus* has an unusual cochlear structure that allows it to hear low frequency sound (Bruns and Burda 1989), and female *Ormia* flies have specialized hearing organs that enable them to locate their calling cricket hosts (Robert et al. 1992). In the fourth chapter I provide evidence that eavesdropping may also be involved in niche partitioning between closely related species by enabling predators to take advantage of different prey. This chapter emphasizes the importance of eavesdropping not only as a foraging strategy but also as one of the many means by which natural selection generates biodiversity.

Together these chapters highlight the extraordinary foraging strategy that is eavesdropping, and its implications for cognition and evolution. This research also contributes to our understanding of how bats perceive the world around them, and I hope inspires further investigation of the question posed in the quote by Nagel (1974) in the introduction, of “what is it like to be a bat”?

# REFERENCES

Adams-Hunt MM, Jacobs LF (2007) Cognition for foraging. In: Stephens DW, Brown JS, Ydenberg RC (eds) Foraging: behavior and ecology. University of Chicago Press, Chicago, pp 105-138

Akre KL, Farris HE, Lea AM, Page RA, Ryan MJ (2011) Signal perception in frogs and bats and the evolution of mating signals. Science 333:751-752

Anderson ME, Racey PA (1991) Feeding behavior of captive long-eared bats, *Plecotus auritus*. Anim Behav 42:489-493

Andersson MB (1994) Sexual selection. Princeton University Press, Princeton NJ
Arak A, Eiriksson T (1992) Choice of singing sites by male bushcrickets (*Tettigonia viridissima*) in relation to signal propagation. Behav Ecol Sociobiol 30:365-372

Arlettaz R (1996) Feeding behaviour and foraging strategy of free-living mouse-eared bats, *Myotis myotis* and *Myotis blythii*. Anim Behav 51:1-11.

Arlettaz R (1999) Habitat selection as a major resource partitioning mechanism between the two sympatric sibling bat species *Myotis myotis* and *Myotis blythii*.

Arlettaz R, Jones G, Racey PA (2001) Effect of acoustic clutter on prey detection by bats. Nature 414: 742-745

Arlettaz R, Perrin N, Hausser J (1997) Trophic resource partitioning and competition between the two sibling bat species *Myotis myotis* and *Myotis blythii*. J Anim Ecol 66:897-911

Arnold SJ (1977) Polymorphism and geographic variation in the feeding behavior of the garter snake *Thamnophis elegans*. Science 4304:676-678.

Bailey WJ, Haythornthwaite S (1998) Risks of calling by the field cricket *Teleogryllus oceanicus*; potential predation by Australian long-eared bats. J Zool Lond 244:505-513

Barnard CJ, Brown CAJ (1985) Risk-sensitive foraging in common shrews (*Sorex araneus* L.). Behav Ecol Sociobiol 16:161-164.

Bates D, Maechler M, Bolker B (2013) lme4: Linear mixed-effects models using S4 classes. R package version 0.999999-2. http://CRAN.R-project.org/package=lme4. Accessed 24 May 2013

Bell G (1982) Behavioral and ecological aspects of gleaning by a desert insectivorous bat *Antrozous pallidus* (Chiroptera: Vespertilionidae). Behav Ecol Sociobiol 10:217–223.

Belwood J, Morris G (1987) Bat predation and its influence on calling behavior in neotropical katydids. Science 238:64.

Bernal XE, Page RA, Rand AS, Ryan MJ (2007) Cues for eavesdroppers: do frog calls indicate prey density and quality? Am Nat 169:409-415.

Bernal XE, Rand AS, Ryan MJ (2006) Acoustic preferences and localization performance of blood-sucking flies (*Corethrella* Coquillett) to túngara frog calls. Behav Ecol 17: 709–715

Bernays EA, Wcislo WT (1994) Sensory capabilities, information processing, and resource specialization. Q Rev Biol 69:187-204

Bogdanowicz W, van den Bussche R, Gajewska M, Postawa T, Harutyunyan M (2009) Ancient and contemporary DNA sheds light on the history of mouse-eared bats in Europe and the Caucasus. Acta Chiropterol 11:289–305

Boul KE, Ryan MJ (2004) Population variation of complex advertisement calls in *P. petersi* and comparative laryngeal morphology. Copeia 2004:624-631.

Boyd R, Richerson PJ (1985) Culture and the Evolutionary Process. University of Chicago Press, Chicago

Boyd R, Richerson PJ (1988) An evolutionary model of social learning: the effects of spatial and temporal variation. In: Zentell TR, Galef BG (eds) Social Learning: Psychological and Biological Perspectives. Lawrence Erlbaum: Hillsdale, NJ, USA pp 29-48

Bruns V, Burda H., Ryan MJ (1989) Ear morphology of the frog-eating bat (*Trachops cirrhosus,* family: Phyllostomidae): Apparent specializations for low-frequency hearing. J Morphol 199:103-118

Buchler ER, Childs SB (1981) Orientation to distant sounds by foraging big brown bats (*Eptesicus fuscus*). Anim Behav 29:428-432

Cade W (1975) Acoustically Orienting Parasitoids: Fly Phonotaxis to Cricket Song. Science 190:1312–1313

Campbell RA (1963) Detection of a noise signal of varying duration. J Acoust Soc Am 35:1732–1737

Cherry EC (1953) Some experiments on the recognition of speech, with one and two ears. J Acoust Soc Am 25:975–979

Clare EL, Lim BK, Fenton MB, Hebert PDN (2011) Neotropical bats: estimating species diversity with DNA barcodes. PLoS One 6, e22648.

Cramer MJ, Willig MR, Jones C (2001) *Trachops cirrhosus*. Mammalian Species: American Society of Mammalogists 656:1-6.

Darwin C (1871) The descent of man and selection in relation to sex. J. Murray, London

Duffy GA, Pike TW, Laland KN (2009) Size-dependent directed social learning in nine-spined sticklebacks. Anim Behav 78:371-375

Dugatkin LA (1992) Sexual selection and imitation: females copy the mate choice of others. Am Nat 139:1384-1389

Dugatkin LA, Godin JGJ (1992) Reversal of female mate choice by copying in the guppy (*Poecilia reticulata*). Proc R Soc B 249:179-184.

Dukas R (1998) Cognitive ecology: the evolutionary ecology of information processing and decision-making. University of Chicago Press, Chicago

Dukas R (2004) Evolutionary biology of animal cognition. Annu Rev Ecol Evol S 35:347-374

Dukas R, Bernays EA (2000) learning improves growth rate in grasshoppers. PNAS 97:2637-2640

Emlen J (1966) The role of time and energy in food preference. American Naturalist
Farris H, Rand AS, Ryan MJ (2002) Auditory grouping in the túngara frog: The roles of complex call components in what and where decisions. J Acous Soc Am 112:2259–2259.

Farris HE, Oshinsky ML, Forrest TG, Hoy RR (2008) Auditory sensitivity of an acoustic parasitoid (*Emblemasoma* sp., Sarcophagidae, Diptera) and the calling behavior of potential hosts. Brain Behav Ecol 72:16-26

Farris HE, Rand AS, Ryan MJ (2002) The effects of spatially separated call components on phonotaxis in túngara frogs: Evidence for auditory grouping. Brain Behav Evol 60:181-188

Farris HE, Rand AS, Ryan MJ (2005) The effects of time, space and spectrum on auditory grouping in túngara frogs. J Comp Physiol A 191:1173-1183

Farris HE, Ryan MJ (2011) Relative comparisons of call parameters enable auditory grouping in frogs. Nat Commun 2:410

Faure PA, Barclay RMR (1992) The sensory basis of prey detection by the long-eared bat, *Myotis evotis*, and the consequences for prey selection. Anim Behav 44:31–39

Faure PA, Barclay RMR (1992) The sensory basis of prey detection by the long-eared bat, *Myotis evotis*, and the consequences for prey selection. Anim Behav 44: 31-39

Faure PA, Hoy PP (2000) The sounds of silence: cessation of singing and song pausing are ultrasound-induced acoustic startle behaviors in the katydid *Neoconocephalus ensiger* (Orthoptera; Tettigoniidae). J Comp Physiol A 186:129-142

Fiedler J (1979) Prey catching with and without echolocation in the Indian False Vampire (*Megaderma lyra*). Behav Ecol Sociobiol 6:155-160

Fisher J, Hinde RA (1949) The opening of milk bottles by birds. Brit Birds 42:347-357

Fowler HG (1987) Field behavior of *Euphasiopteryxdepleta* (Diptera: Tachinidae): phonotactically orienting parasitoids of mole crickets (Orthoptera: Gryllotalpidae: Scapteriscus). J NY Entomol Soc 95:474-480

Galef BG, Giraldeau LA (2001) Social influences on foraging in vertebrates: causal mechanisms and adaptive functions. Anim Behav 61:3-15

Galef BG, Stein M (1985) Demonstrator influence on observer diet preference: analyses of critical social interactions and olfactory signals. Anim Learn Behav 13:31-38

Galef BG, White DJ (1998) Mate-choice copying in Japanese quail, *Coturnix japonica*. Anim Behav 55:545-552

Galef BG (2009) Strategies for social learning: testing predictions from formal theory. Adv Stud Behav 39:117-151

Galef BG, Dudley KE, Whiskin EE (2008) Social learning of food preferences in ‘dissatisfied’ and ‘uncertain’ Norway rats. Anim Behav 75:631-637

Gaudet CL, Fenton MB (1984) Observational learning in three species of insectivorous bats (Chiroptera). Anim Behav 32:385-388

Giraldeau LA, Valone TJ, Templeton JJ (2002) Potential disadvantages of using socially acquired information. Philos T Roy Soc B 357:1559-1566

Goerlitz HR, Greif S, Siemers BM (2008) Cues for acoustic detection of prey: insect rustling sounds and the influence of walking substrate. J Exp Biol 211:2799-2806

Gray DA, Banuelos C, Walker SE, Cade WH, Zuk M (2007) Behavioural specialization among populations of the acoustically orienting parasitoid fly *Ormia ochracea* utilizing different cricket species as hosts. Anim Behav 73:99-104

Greenberg R (1983) The role of neophobia in determining the degree of foraging specialization in some migrant warblers. Am Nat 122:444-453

Grüter C, Ratnieks FLW (2011) Honeybee foragers increase the use of waggle dance information when private information becomes unrewarding. Anim Behav 81:949-954

Grüter C, Czaczkes TJ, Ratnieks FLW (2011) Decision making in ant foragers (*Lasius niger*) facing conflicting private and social information. Behav Ecol Sociobiol 65:141-148

Haynes KF, Yeargan KV (1999) Exploitation of intraspecific communication systems: illicit signalers and receivers. Ann Entomol Soc Am 92:960–970

Hedrick AV, Richert SE (1989) Genetically-based variation between two spider populations in foraging behavior. Oecologia 4:533-539

Heller K-G (1988) Die Bioakustik der Europäischen Laubheuschrecken. J Margraf, Weikersheim, Germany

Hofstede ter H, Ratcliffe J, Fullard J (2008) The effectiveness of katydid (*Neoconocephalus ensiger*) song cessation as antipredator defence against the gleaning bat *Myotis septentrionalis*. Behavioral Ecology and Sociobiology 63:217–226

Højsgaard S, Halekoh U, Yan J (2006) The R Package geepack for Generalized Estimating Equations. J Stat Softw 15:1-11

Hoke KL, Burmeister SS, Fernald RD, Rand AS, Ryan MJ, Wilczynski W (2004) Functional mapping of the auditory midbrain during mate call reception. J Neurosci 24:11264-11272

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biometrical J 50:346-363

Hubbell SP (2001) The Unified Neutral Theory of Biodiversity and Biogeography. Princeton University Press, Princeton NJ

Ibáñez R, Rand AS, Jamarillo C (1999) The amphibians of Barro Colorado Nature Monument, Soberania National Park and adjacent areas. Mizrachi and Pujol, S.A., Panamá.

Ibáñez R, Rand AS, Ryan MJ, Jaramillo CA (1999) Vocalizations of frogs and toads from Barro Colorado Nature Monument , Soberanía National Park and adjacent areas [CD]. Panamá: Fundación Natura, Circulo Herpetologico de Panamá & Smithsonian Tropical Research Institute.

Ings TC, Raine NE, Chittka L (2009) A population comparison of the strength and persistence of innate colour preference and learning speed in the bumblebee *Bombus terrestris*. Behav Ecol Sociobiol 63:1207–1218

Jones PL, Page RP, Hartbauer M, Siemers BM (2011) Behavioral evidence for eavesdropping on prey song in two Palearctic sibling bat species. Behav Ecol Sociobiol 65:333–340

Jones PL, Ryan MJ, Flores V, Page RA (2013) When to approach novel prey cues? Social learning strategies in frog-eating bats. Proc R Soc B 280:20132330

Kalko EKV, Friemel D, Handley CO, Schnitzler HU (1999) Roosting and foraging behavior of two Neotropical gleaning bats, *Tonatia silvicola* and *Trachops cirrhosus* (Phyllostomidae). Biotropica 31:344-353

Kelly A, Goodwin S, Grogan A, Mathews F (2008) Post-release survival of hand-reared pipistrelle bats (*Pipistrellus* spp). Anim Welfare 17:375-382

Kendal JR, Rendell L, Pike TW, Laland KN (2009) Nine-spined sticklebacks deploy a hill-climbing social learning strategy. Behav Ecol 20:238-244

Kendal RL, Coolen I, Laland KN (2009) Adaptive trade-offs in the use of social and personal information. In *Cognitive Ecology II* (eds R. Dukas & J.M. Ratcliffe), pp. 249-271. Chicago, USA: University of Chicago Press.

Keuper A, Weidemann S, Kalmring K, Kaminski D (1988) Sound production and sound emission in seven species of European Tettigoniids. Part I. The different parameters of the song; their relation to the morphology of the bushcricket. Bioacoustics 1:31-48

Kolb A (1961) Sinnesleistungen einheimischer Fledermäuse bei der Nahrungssuche und Nahrungsauswahl auf dem Boden und in der Luft. Z vergl Physiol 44:550–564

Lakes-Harlan R, Stölting H, Stumpner A (1999) Convergent evolution of insect hearing organs from a preadaptive structure. Proc R Soc Lond B 266:1161-1167

Laland KN, Plotkin HC (1991) Excretory deposits surrounding food sites facilitate social learning of food preferences in Norway rats. Anim Behav 41:997-1005

Laland KN (2004) Social learning strategies. Learn Behav 32:4-14

Lang AB, Kalko EKV, Römer H, Bockholdt C, Dechmann DKN (2006) Activity levels of bats and katydids in relation to the lunar cycle. Oecologia 146: 659–666

Lloyd JE, Wing SR (1983) Nocturnal aerial predation of fireflies by light-seeking fireflies. Science 222:634-635

MacArthur RH, Pianka ER (1966) On optimal use of a patchy environment. Am Nat 100:603–609

Morris GK, Mason AC, Wall P, Belwood JJ (1994) High ultrasonic and tremulation signals in neotropical katydids (Orthoptera: Tettigoniide). J Zool 233:129-163

Nagel T (1974) What is it like to be a bat? Philos Rev 83:435–450

Neuhauser M (2004) Testing whether any of the significant tests within a table are indeed significant. Oikos 106:409–410

Nyberg DN (1971) Prey capture in the largemouth bass. Amer Midl Nat 86:128-144

Page RA, Ryan MJ (2005) Flexibility in assessment of prey cues: frog-eating bats and frog calls. Proc R Soc B 272:841-847

Page RA, Ryan MJ (2006) Social transmission of novel foraging behavior in bats: anuran calls and their referents. Curr Biol 16:1201–1205

Page RA, Ryan MJ (2008) The effect of signal complexity on localization performance in bats that localize frog calls. Anim Behav 76:761-769

Page RA, Ryan MJ, Bernal XE (in press) Be loved, be prey, be eaten. In: Yasukawa K (ed) Animal Behavior, 3 vols, Praeger.

Panger MA, Perry S, Rose L, Gros-Louis J, Vogel E, Mackinnon KC, Baker M (2002) Cross-site differences in foraging behavior of white-faced capuchins (*Cebus capucinus*). Am J Phys Anthropol 119:52-66

Peake TM (2005) Eavesdropping in communication networks. In: McGregor PK (ed) Animal communication networks. Cambridge University Press, Cambridge, pp 13-37

Pereira MJR, Rebelo H, Rainho A, Palmeirim JM (2002) Prey selection by *Myotis myotis* (Vespertilionidae) in a Mediterranean region. Acta Chiropterol 4:183–193

Pianka ER (1981) Competition and niche theory. In: May R (ed) Theoretical ecology: Principles and Applications. Sinauer Associates, Sunderland MA, pp 167–196

Pike TW, Laland KN (2010) Conformist learning in nine-spined sticklebacks' foraging decisions. Biol Lett 6:466–468

Pike TW, Kendal JR, Rendell LE, Laland KN (2010) Learning by proportional observation in a species of fish. Behav Ecol 21:570-575

Pintor LM, Sih A (2009) Differences in growth and foraging behavior of native and introduced populations of an invasive crayfish. Biol Invasions 11:1895-1902

Pollak G, Henson OW Jr., Novick A (1972) Cochlear microphonic audiograms in the "pure tone" bat *Chilonycteris parnellii parnellii*. Science 176:66-68

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

R Core Team (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Raine NE, Chittka L (2008) The correlation of learning speed and natural foraging success in bumblebees. Proc R Soc B 275:803-808

Rand AS, Ryan MJ (1981) The adaptive significance of a complex vocal repertoire in a neotropical frog. Z Tierpsychol 57:209–214

Ratcliffe JM, ter Hofstede HM (2005) Roosts as information centres: social learning of food preferences in bats. Biol Lett 1:72-74

Rendell L, Fogarty L, Hoppitt WJE, Morgan TJH, Webster MM, Laland KN (2011) Cognitive culture: theoretical and empirical insights into social learning strategies. Trends Cogn Sci 15:68-76

Richards LA, Coley PD (2007) Seasonal and habitat differences affect the impact of food and predation on herbivores: a comparison between gaps and understory of a tropical forest. Oikos 116: 31–40

Richards LA, Windsor DM (2007) Seasonal variation of arthropod abundance in gaps and the understory of a lowland moist forest in Panama. Journal of Tropical Ecology 23:169-176

Robert D, Amoroso J, Hoy RR (1992) The evolutionary convergence of hearing in a parasitoid fly and its cricket host. Science 258:1135-1137

Roberts SC, Gosling LM, Thornton EA, McClung J (2001) Scent marking by male mice under the risk of predation. Behav Ecol 12:698-70

Rogers A (1988) Does biology constrain culture? Am Anthropol 90:819-813

Ruedi M, Mayer F (2001) Molecular systematics of bats in the genus *Myotis* (Vespertilionidae) suggests deterministic ecomorphological convergences. Mol Phylogenet Evol 21:436-448

Russo D, Jones G, Arlettaz R (2007) Echolocation and passive listening by foraging mouse-eared bats *Myotis myotis* and *M. bythii*. J Exp Biol 210:166-176

Ryan M, Tuttle M (1987) The role of prey-generated sounds, vision, and echolocation in prey localization by the African bat *Cardioderma cor* (Megadermatidae). J Comp Physiol A 161:59–66

Ryan MJ (1980) Female mate choice in a Neotropical frog. Science 209:523-525.

Ryan MJ (1985) The túngara frog, a study in sexual selection and communication. University of Chicago Press, Chicago

Ryan MJ, Rand AS (2003) Sexual selection in female perceptual space: how female túngara frogs perceive and respond to complex population variation in acoustic mating signals. Evolution 57:2608–2618

Ryan MJ, Tuttle MD (1983) The ability of the frog-eating bat to discriminate among novel and potentially poisonous frog species using acoustic cues. Anim Behav 31:827-833.

Ryan MJ, Tuttle MD (1987) The role of prey-generated sounds, vision, and echolocation in prey localization by the African bat *Cardioderma cor* (Megadermatidae). J Comp Physiol A 161:59-66

Ryan MJ, Tuttle MD, Rand AS (1982) Bat predation and sexual advertisement in a neotropical anuran. Am Nat 119:136-139

Safi K., Siemers BM (2010) Implications of sensory ecology for species coexistence: biased perception links predator diversity to prey size distribution. Evol Ecol 24:703-713

Sakaguchi KM, Gray DA (2011) Host song selection by an acoustically orienting parasitoid fly exploiting a multispecies assemblage of cricket hosts. Anim Behav 81:851-858

Schaub A, Ostwald J, Siemers BM (2008) Foraging bats avoid noise. J Exp Biol 211:3174-3180

Schlag K (1998) Why imitate, and if so, how? J Econ Theory 78:130–156

Schlupp I, Marler CA, Ryan MJ (1994) Benefit to male sail fin mollies of mating with heterospecific females. Science 263:373-374

Schluter D (2000) The Ecology of Adaptive Radiation. Oxford University Press, Oxford

Schulze W, Schul J (2001) Ultrasound avoidance behaviour in the bushcricket *Tettigonia viridissima* (Orthoptera: Tettigoniidae). J Exp Biol 204:733-740

Sherwin CM, Heyes CM, Nicola CJ (2002) Social learning influences the preferences of domestic hens for novel food. Anim Behav 63:933-942

Sherry DW, Mitchell JB (2007) Neuroethology of foraging. In: Stephens DW, Brown JS, Ydenberg RC (eds) Foraging: behavior and ecology. University of Chicago Press, Chicago, pp 61-102

Shettleworth SJ (1998) Cognition, evolution, and behavior. Oxford University Press, New York

Siemers BM, Güttinger R (2006) Prey conspicuousness can explain apparent prey selectivity. Curr Biol 16:157-159

Siemers BM, Schnitzler HU (2004) Echolocation signals reflect niche differentiation in five sympatric congeneric bat species. Nature 429:657-661

Siemers B, Swift S (2006) Differences in sensory ecology contribute to resource partitioning in the bats *Myotis bechsteinii* and *Myotis nattereri* (Chiroptera: Vespertilionidae). Behav Ecol Sociobiol 59:373–380

Spangler HG (1984) Silence as a defense against predatory bats in two species of calling insects. Southwest Nat 29:481-488

Stephens DW (1981) The logic of risk-sensitive foraging preferences. Anim Behav 29:628-629

Stephens DW, Brown JS, Ydenberg RC (2007) Foraging: behavior and ecology. University of Chicago Press

Stowe MK, Turlings TCJ, Loughrin JH, Lewis WJ, Tumlinson JH (1995) The chemistry of eavesdropping, alarm, and deceit. Proc Natl Acad Sci USA 92:23-28

Stumpner A, Allen GR, Lakes-Harlan R (2007) Hearing and frequency dependence of auditory interneurons in the parasitoid fly *Homotrixa alleni* (Tachinidae: Ormiini). J Comp Physiol A 193:113-125

Swift SM, Racey PA (2002) Gleaning as a foraging strategy in Natterer's bat *Myotis nattereri*. Behav Ecol Sociobiol 52:408-416

Tandler B, Phillips CJ, Nagato T (1996) Histological convergent evolution of the accessory submandibular glands in four species of frog-eating bats. Eur J Morphol 34:63-168

ter Hofstede HM, Kalko EKV, Fullard JH (2010) Auditory-based defense against gleaning bats in neotropical katydids (Orthoptera: Tettigoniidae). J Comp Physiol A 196:349-358

ter Hofstede HM, Ratcliffe JM, Fullard JH (2008) The effectiveness of katydid (*Neoconocephalus ensiger)* song cessation as antipredator defense against the gleaning bat *Myotis septentionalis*. Behav Ecol Sociobiol 63: 217-226

Thornton A (2008) Social learning about novel foods in young meerkats. Anim Behav 76:1411-1421

Tilman D (2004) Niche tradeoffs, neutrality, and community structure: a stochastic theory of resource competition, invasion, and community assembly. Proc Natl Acad Sci USA 101:10854–10861

Trillo PA, Athanas KA, Goldhill DH, Hoke KL, Funk WC (2013) The influence of geographic heterogeneity in predation pressure on sexual signal divergence in an Amazonian frog species complex. J Evolution Biol 26:216-222.

Tuttle M, Ryan M (1981) Bat predation and the evolution of frog vocalizations in the Neotropics. Science 214:677–678

Tuttle MD, Ryan MJ, Belwood JJ (1985) Acoustical resource partitioning by two species of phyllostomid bats (*Trachops cirrhosus* and *Tonatia sylvicola*). Anim Behav 33:1369-1370

Tuttle MD, Taft LK, Ryan MJ (1982) Evasive behavior of a frog in response to bat predation. Anim Behav 30:393-397

van Bergen Y, Coolen I, Laland KN (2004) Nine-spined sticklebacks exploit the most reliable source when public and private information conflict. Proc R Soc B 271:957-962

van Schaik C, Ancrenaz M, Borgen G, Galdikas B, Knott CD, Singleton I, Suzuki A, Utami SS, Merrill M (2003) Orangutan cultures and the evolution of material culture. Science 299:102-105

Venables WN, Ripley BD (2002) Modern Applied Statistics with S. Fourth Edition. Springer, New York

Visalberghi E, Fragaszy D (1995) The behaviour of capuchin monkeys, *Cebus apella*, with novel food: the role of social context. Anim Behav 49:1089-1095

Wagner WE Jr (1996) Convergent song preferences between female field crickets and acoustically orienting parasitoid flies. Behav Ecol 7:279-28

Wagner, W. E. Jr (2011) Direct benefits and the evolution of female mating preferences: conceptual problems, potential solutions, and a field cricket. Adv Stud Behav 43:273-319

Walker TJ (1993) Phonotaxis in female *Ormia ochracea* (Diptera: Tachinidae), a parasitoid of field crickets. J Insect Behav 6:389-410

Webster MM, Laland KN (2011) Reproductive state affects reliance on public information in sticklebacks. Proc R Soc B 278:619-627

Whiten A, Goodall J, McGrew WC, Nishida T, Reynolds V, Sugiyama Y, Tutin CE, Wrangham RW, Boesch C (1999) Cultures in chimpanzees. Nature 399:682–685

Whiten A, Horner V, de Waal FBM (2005) Conformity to cultural norms of tool use in chimpanzees. Nature 437:737-740

Wilczynski W, Rand AS, Ryan MJ (2001) Evolution of calls and auditory tuning in the *Physalaemus pustulosus* species group. Brain Behav Evol 58:137-151

Wilkinson GS (1992) Information transfer at evening bat colonies. Anim Behav 44:501-518

Wolda H (1978) Seasonal fluctuations in rainfall, food and abundance of tropical insects. J Anim Ecol 47:369-381