Anti-predator behavior in Neotropical bats

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Anti-predator behavior in Neotropical bats

A Dissertation Presented for the
Doctor of Philosophy
Degree
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Melquisedec Gamba-Rios
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DEDICATION

To my parents and Mafe for their unconditional support.
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ABSTRACT

Predation is one of the main drivers changing animal populations and communities, as well as playing a key role in animal behavior and ultimately on the evolutionary processes of the species. Pressure from lethal and non-lethal predation has profound consequences on the life-history of animals, and studying the mechanisms under which predation operates is fundamental to understanding many natural processes and their implication on the survival and conservation of species. I used two different bat species as study systems to understand three main questions. How do prey detect and discriminate predators? Which behaviors do prey use for predator avoidance? And, what is the cost of antipredator behaviors for communication and sociality? Using the Spix’s disk-winged bat (Thyroptera tricolor), I documented the first interspecific echolocation call recognition in bats in the context of predator-prey interaction. When predator calls are detected, bats display antipredator behaviors that disrupt social communication and social cohesion. Additionally, I showed that the tent-making bat (Dermanura watsoni) uses auditory cues as the first filter to rate predation risk and, with escalating risk of predation, visual and physical cues play a vital role in antipredation behavior. Finally, I reviewed how wild animals perceive humans as predators and the impacts of nature-based tourism on bats. These impacts may occur mainly at bat roosts during the daytime, having significant consequences on key moments in life history traits involving vigilance, sleep and social behaviors.
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INTRODUCTION

In nature, most species are preyed upon by multiple other species, a fact which has led to numerous studies on predator-prey interactions and their effects at different scales. Predation is one of the drivers of population dynamics for both prey and their predators (MacLulich 1937, Huffaker 1958, Gilg et al. 2003, Turchin 2003). Furthermore, predation has profound effects at the community level, causing major shifts in community structure (Paine 1974). Ultimately, these changes at population and community levels can drive changes in the ecosystem through both lethal and non-lethal effects of predation (Parmenter et al. 2003, Croll et al. 2005). As a consequence, predation is a major driver in nature and evolution, shaping a suite of traits and behavioral strategies in prey organisms to prevent premature death. Therefore, selection should favor sensory mechanisms enabling organisms to detect the presence of predators and evaluate predation risk. Animals use different cues to recognize threats, such as visual, auditory, chemical or a combination of these sensory modalities (Hauser and Wrangham 1990, Hauser and Caffrey 1994, Werner and Peacor 2003, Brinkerhoff et al. 2005, MacLean and Bonter 2013). These sensory modalities are fundamental for predator recognition and for displaying the correct set of anti-predator behaviors (Caro 2005).

In the late nineteenth century, the first zoologists that attempted to understand antipredator strategies focused primarily on animal coloration (Poulton 1890, Beddard 1892, Thayer and Thayer 1909). The work of Hugh B. Cott in the 1940’s continued these efforts on the importance of coloration and camouflage and their military applications (Cott 1940). In the 1950’s Karl von Frisch, Konrad Lorenz and Nikolaas Tinbergen formed the springboard for modern behavioral ecology with their detailed behavioral studies of birds and insects. Since then, both theoretical
and empirical advances have been made in understanding the influence of predators on the behavior of their prey, covering a wide range of topics, such as sociality (Krause et al. 2002, Caro 2005), animal personalities (Sih et al. 2004), foraging behavior (Lima and Dill 1990), physiology (Creel et al. 2009) and sensory mechanisms (Fernández-Juricic et al. 2004).

Bats are one of the most diverse groups of mammals, with over 1400 species worldwide and comprising about 20% of all mammalian species (Simmons 2005). However, this group is poorly represented in the literature on anti-predator behavior. This could likely be due to the nocturnal habits and the lack of specialized predators on bats. Predation on bats seems to be opportunistic, with records of bats being preyed upon by many mammals (Jones and Manning 1989), birds (Fenton and Fleming 1976), reptiles (Davis et al. 1962), amphibians (Kinsey 1961), fish (Ingles 1954) and invertebrates (Rice 1957). Most of the documented studies of predation occur as bats emerge from caves (Stager 1941, Gillette and Kimbrough 1970, Rodriguez-Duran and Lewis 1985, McCracken et al. 1986, Sparks et al. 2000, Lee and Kuo 2001). However, for non-cave roosting bats, predator-driven behaviors are poorly understood or are unknown. In foliage-roosting bats, the ability to assess predation risk could be particularly essential for survival, because leaving the roost during the daylight hours could increase the predation rate 100-1000 times more than at night (Speakman 1991, Speakman 1995).

The work I present here is intended to enlighten three main topics. First, for animals to display effective antipredator behavior, they must rely on the detection and discrimination of cues from other animals. Understanding how prey detect and discriminate predators is a fundamental mechanism for any antipredator strategy. Second, after a predator is detected, what are the
behaviors that prey use for predator avoidance? Finally, antipredator behaviors are intended to avoid or elude predators, but these behaviors may unintentionally affect both intra- and interspecific communication. Therefore, it is crucial to understand the cost of these antipredator behaviors for prey communication and sociality. To address these main topics, I used experimental methods and literature review, divided into three chapters in this document.

In the first chapter, I assess detection and discrimination of direct and indirect cues from predators, both experimentally in situ. Also, I test the bats’ decision-making process using multiple playback cues (acoustic, visual and physical). In addition to these experimental methods, I monitor which animal species and potential predators move around the roosts. I work with the Thomas’s fruit-eating bat (*Dermanura watsoni*) as a study system. D. watsoni is a frugivorous bat that uses modified leaves as roosts (Figure 1), and more than 40 plant species have been recorded to be used as roosts (Kunz and Lumsden 2003), with different roost architectures (e.g. bifid, umbrella, apical, boat, and boat-apical) (Rodríguez-Herrera et al. 2007). Bats modify the leaves by partially cutting the midrib, veins and surrounding tissue of specific leaves, and, as a consequence, the lamina of the leaf collapse to give the shape of a “tent”. Depending on the plant species, these tents can be found from around one meter to several meters above the ground,
Figure 1. Thomas’s fruit-eating bat (*Dermanura watsoni*). Bats roost under a modified leaf and four individuals can be observed here (three adults and one pup). Bites along the midrib of the leaf can be seen. These bites cause the lamina on both sides to collapse, forming the tent. Small holes on the lamina function as landing grips for the bats. Photo: M Gamba-Rios.
giving bats a panoramic view, which allows them to evaluate possible predation risks coming from below (Kunz and Lumsden 2003). Since most of tents are built on flexible plants or leaves, any physical movement on the plant by a potential predator provides the bats with an alert system. Some tent-making bat species use separate night roosts, which may help them to reduce conspicuity from the accumulation of feces, seeds, and fruit parts under the roost, that could attract predators (Threlfall et al. 2013). Having separate day and night roosts may also decrease exposure while they are feeding (Brooke 1990, Charles-Dominique 1993). In addition to bats, wasps can use tents by making nests that are similar in size and shape to roosting bats under the tents, and it is suggested that bats may be mimicking wasp nests as another antipredator strategy (Figure 2) (Rodríguez-Herrera et al. 2007).

The second chapter experimentally addresses the ability of bats that are preyed upon by other bats to discriminate between echolocation calls of predators and non-predators. Additionally, I test the impact of strategies to reduce predation risk on communication and sociality. In this chapter I work with the Spix’s disk-winged bat (*Thyroptera tricolor*) in their roost (Figure 3). This species provides a study system that relies completely on acoustic cues to recruit members and evaluate predation risk. *T. tricolor* roosts inside the developing furled leaves of plants, mainly of the genus *Heliconia*, *Musa*, and *Calathea* (Findley and Wilson 1974). Furled leaves are a highly ephemeral roost, remaining within the preferred size for bats between 8 to 16 hours and forcing individuals to locate a new roost every day (Vonhof and Fenton 2004). Despite frequent roost switching, *T. tricolor* maintains a very stable composition of highly related
**Figure 2.** Thomas’s fruit-eating bat (*Dermanura watsoni*) on the left side and wasp nest on the right side. It has been suggested that bats may use the similarity to the wasp nest when roosting as an antipredator strategy. A) Photograph taken without flash, under natural light. B) Photograph taken with flash. Photos: B. Rodríguez-Herrera and Figure taken from Rodríguez-Herrera et al. 2007.
Figure 3. Spix’s disk-winged bat (*Thyroptera tricolor*), emerging from a furled leaf roost. Photo: M Gamba-Rios.
social groups (Chaverri 2010, Buchalski et al. 2014). These social groups are composed of varying proportions of philopatric males and females, and group members remain within their natal territory and social group for several years (Chaverri 2010, Chaverri and Kunz 2011, Buchalski et al. 2014).

This species maintains its highly cohesive social aggregation by using a system of contact calls (Chaverri et al. 2012), which helps to recruit group members to the roost. A specialized social call system suggests benefits for forming groups while roosting. Thermoregulation has been proposed as a benefit to decrease energetic expenditure in animal aggregations (Scantlebury et al. 2006, Willis and Brigham 2007). However, *T. tricolor* inhabits warm lowland forest, which infers that thermoregulation may not be a major advantage to forming groups. Rapid response to predator attacks and reduced individual investment on vigilance could be important for the recruitment of group members. This needs to be tested in *T. tricolor*, especially because it has been suggested that prey respond faster to attacks when individuals rest in groups compared to solitarily (Semeniuk and Dill 2004). Allofeeding also could be a benefit for maintaining group cohesion, and it been observed in several bat species (Wilkinson 1984, 1992, Geipel et al. 2013), although, this kind of behavior has not been observed yet in *T. tricolor*.

In the final chapter, I highlight the need to study the impacts of nature-based tourism on bats, a poorly known issue with implications on conservation and management in wildlife settings. Visitors to wildlife areas can have both direct and indirect impacts on bats’ behaviors, such as, vigilance, sleep, roost and habitat selection. As a consequence, these human-induced disturbances can affect fitness, decreasing or extirpating populations in disturbed areas. These
Impacts may occur mainly at bat roosts during the daytime, having significant consequences on key life history traits and social behaviors. Understanding these impacts and processes are fundamental, especially because nature-based tourism has become one of the largest industries worldwide.

Predation and antipredation strategies in bats are not well understood. Documenting the behavioral ecology of foliage-roosting bats, as revealed in this body of research, enlightens a field which has deep ecological and evolutionary consequences on nature.
References


Cott, H. B. 1940. Adaptive coloration in animals. Methuen; London.


Poulton, E. B. 1890. The colours of animals: their meaning and use, especially considered in the case of insects. D. Appleton.


CHAPTER I
BEHAVIORAL RESPONSE TO PREDATOR CUES IN TENT-MAKING BATS
My consistent use of “we” throughout this chapter is in reference to my co-authors and myself. I was the primary contributor to this work, which involved the following tasks: (1) development of project design and all data collection, (2) measurement of acoustic and video data and statistical analysis, (3) gathering and interpretation of the relevant literature, and (4) all of the writing.

Abstract

Hearing may be an important sensory modality for detecting predators, particularly in organisms such as bats that rely heavily on acoustic cues for navigation and foraging. To date there is little evidence that predator auditory cues influence the behavior of bats, despite the fact that correct assessment of predation risk, coupled with an adequate behavioral response, should increase survival. Since bats spend half of their lives in day roosts, predatory events that occur at or near roosts should play a major role in driving their ecology and behavior. This study examines the response of bats to several auditory and physical predator cues at their diurnal roosts. We hypothesize that bats’ response increases with the perceived risk provided by the presented stimulus. Using video cameras and ultrasonic microphones, we first recorded the diurnal activity of 3 control groups of the tent-making bat *Dermanura watsoni* (Phyllostomidae: Stenodermatinae) to obtain baseline information regarding normal roosting behavior. We then used auditory playback sounds of a variety of possible predators, conspecific acoustic cues, and sounds likely associated with the approach of predators. Additionally, we used the movement of branches as physical cues next to each roost, measuring bat responses in 24 different groups. As expected, the response of the groups escalated as the simulated risk of predation increased. Tent-making bats use acoustic cues as the first stage in predation risk assessment. As the predation risk increased, bats transitioned to a wakefulness state and used visual cues to have a more reliable evaluation of the risk, to ultimately determine when to flee from the roost.
Introduction

The lethal effects of predator-prey interactions affect population size and reproductive success of prey and predators. These effects are a focus of empirical and theoretical study and form a basis of species interactions and community dynamics (Murdoch et al. 2003). However, non-lethal effects of predation also may have large impacts on ecological systems by altering behavior, habitat use and community structure of the prey (Lima 1998, Thomson et al. 2006, Creel et al. 2007). To decrease their risk of predation, prey can alter phenotypic traits including changes in development, physiology, morphology, or behavior. These changes, or anti-predator strategies, can be triggered either by direct cues produced by the predator, including visual, auditory or chemical stimuli (Werner and Peacor 2003, Brinkerhoff et al. 2005, Valeix et al. 2009, Breviglieri et al. 2013, MacLean and Bonter 2013) or indirect cues involving any perceived environmental factor that correlates with the risk of predation (Thorson et al. 1998). As a consequence, changes in real or perceived predation risk may affect animal behavior (Caro 2005, Lima and O'Keefe 2013).

Bats are poorly represented in the literature on anti-predator behavior, likely due to the difficulties of observing these highly mobile and nocturnal animals (Lima and O'Keefe 2013). For bats, most literature on predation involves observations of raptors, owls, snakes and raccoons preying upon bats emerging from caves (Stager 1941, Rodriguez-Duran and Lewis 1985, McCracken et al. 1986, Sparks et al. 2000, Lee and Kuo 2001). However, in the Tropics where many bats roost in foliage in small groups that are scattered over the landscape, predator-driven behavior in bats has received very little attention and is thus poorly understood. Indeed, predation may be less important for the survival of cave-roosting bats than for foliage-roosting
bats which roost in comparatively more exposed locations and could be easy prey for any predator that can find and reach them (Boinski and Timm 1985, Lima and O'Keefe 2013). The ability to assess predation risk within foliage roosts could also have as yet uninvestigated effects on sleep behaviors in bats.

Further, because bats’ hearing is highly developed, we hypothesize that auditory cues will be especially important for detecting predation risk by foliage-roosting bats. Studies have used owl calls to induce responses by bats to the risk of predation, but with ambiguous results (Kalcounis and Brigham 1994, Petrželková and Zukal 2001, Baxter et al. 2006, Breviglieri et al. 2013). These studies were made during the emergence of bats from caves or during foraging activities, and none focused on anti-predator behaviors within the roost. Because bats spend more than half of their lives in their roosts, events associated with roosting play a major role in the ecology and the evolution of bats (Kunz and Lumsden 2003).

*Dermanura watsoni* is among the most versatile of tent-making bat species in the numbers of plant species used to build roosts, roost architecture types, and habitats occupied (Kunz and Lumsden 2003, Rodriguez-Herrera et al. 2007). Here, we investigate the behavioral responses to predation risk induced by different predator cues, including auditory and physical cues, at the diurnal roosts of *D. watsoni* in mature and secondary forest in Costa Rica. We address two main questions. First, do bats base their decision-making process only on acoustic cues? Second, does *D. watsoni* discriminate between conspecific and heterospecific acoustic cues? We show that auditory cues may be the first filter to rate predation risk and, with escalating risk of predation, visual and physical cues may play a vital role in anti-predation behavior in tent-making bats.
Dermanura watsoni discern between conspecific and heterospecific calls, and the behavioral response to heterospecific calls is species specific.

Material methods

(a) Study Areas

We conducted our study in two different locations, Piro Biological Station and Hacienda Baru National Wildlife Refuge, representing different forest succession stages in Costa Rica. Piro Biological Station consists of 1700ha of tropical rainforest on the Osa Peninsula. Most of this area represents an advanced succession, mature forest and is located in the buffer zone of Corcovado National Park with 45000ha of tropical rainforest. The experiments simulating predator and non-predator cues at this station were conducted during June - August 2014, and camera traps were left within this station from June 2014 to July 2015 to record animal-roost interactions. Hacienda Barú National Wildlife Refuge is located on the southern Pacific Coast and has 300ha of tropical rainforest; most of this area represents an early-middle succession, secondary forest surrounded by African oil palm in the northwest and the town of Dominical in the southeast. The experiments at this station were conducted during July - October 2015.

(b) Study species

Thomas’s fruit-eating bat, D. watsoni, (Phyllostomidae: Stenodermatinae) is distributed from central Mexico to northern South America. This small bat (approximately 11 g) feeds primarily
on fruits, but also consumes insects and pollen (LaVal and Rodriguez-H 2002). *D. watsoni* use modified leaves (or “tents”) as roosts, and use more plant species for roosting than are known for any other tent-making bat species (Kunz and Lumsden 2003). The Central American squirrel monkey, *Saimiri oerstedii*, is currently the only predator species reported to recognize leaves modified by tent-making bats and to actively search the tents for prey (Boinski and Timm 1985).

(c) *Animal-roost interactions*

Interactions at tents were recorded using five passive infra-red camera traps (Bushnell 8MP Trophy Cam HD) set a distance of four to six meters from leaves modified as tents by *D. watsoni*. Each camera was set to record 60 seconds of high definition (HD) video, when motion triggered the camera. If motion continued to activate the camera, recording continued after a one second interval, to record any possible interactions with the roost. Cameras were operated over one full year in the mature forest area, with each camera left in front of a single tent for one month, and then relocated to a different tent within the same habitat type. We avoided using the traditional trail-side camera trap setup along trails or paths, since animals using the trails to move between areas could trigger the cameras without interacting with the roosts, thus potentially inflating the data. The videos were filtered to ensure that occurrences were independent; i.e., cases where the same animal stayed around the tent for up to an hour were counted as single event. To minimize bias in the identification process, all videos were examined by two of the authors independently.
(d) Roost behavior

To investigate diurnal behavior of bats at the roost, we recorded bat activity within tents between 8 AM and 5 PM using video (Sony Handycam, Model HDR-XR160) and still cameras (Canon 60D, lens Canon EF-S 18-200 mm 1:3.5-5.6 IS, lens Sigma 150-600 mm 1:5-6.3 DG). Video was synchronized with acoustic recording of the bats’ echolocation calls. Behaviors, such as apparent sleep, alertness, and grooming, were subsequently extracted from the camera recordings, and the number and duration of these behaviors were recorded.

(e) Behavioral response to different cues

We used playback of sounds, including 1) acoustic cues of known predators of bats, 2) acoustic cues of conspecifics, 3) sounds likely associated with the approach of predators, and 4) white noise, to observe whether bats respond to general sounds or specifically to auditory cues of predators (Figure 4). We used an Avisoft UltraSoundGate Player and a broadband loudspeaker (Ultrasonic Omnidirectional Dynamic Speaker Vifa, Avisoft Bioacoustics, Berlin, Germany) to play 30 second intervals of the different sounds. The predator cues or sounds associated with predators were as follows: 1. social calls of the predatory birds Ciccaba nigrolineata (black and white owl), Pulsatrix perspicillata (spectacled owl) and Lophostrix cristata (crested owl); 2. distress and echolocation calls of the predatory bat Vampyrus spectrum (spectral bat); and 3. social calls of Saimiri oerstedii (squirrel monkey), a known tent-making bat predator. We also tested the response to the echolocation and distress calls of conspecifics. Additionally, we used the sound of twigs and leaves breaking to simulate an animal approaching the roost from the ground. For a physical cue, we simulated an animal approaching from the branches by tying a 6
Figure 4. Illustration of a roost of Thomas’s fruit-eating bat (*Dermanura watsoni*) and some of the different cues tested during the experiments. Light grey shadow under the tent represents the field of view of the bats under the roost: Tent = modified leaf used as a roost by *D. watsoni*; (A) owls, *Ciccaba nigrolineata* (black and white owl), *Pulsatrix perspicillata* (spectacled owl) and *Lophostrix cristata* (crested owl); (B) *Saimiri oerstedii* (squirrel monkey); (C) predatory bat *Vampyrum spectrum* (spectral bat); (D) conspecifics calls; (E) sound of twigs and leaves breaking to simulate an animal approaching the roost from the ground; (F) moving of branches, simulating an animal approaching from above the tent. Additionally, (G) *Dasyprocta punctata* (Central American agouti) was the most frequent species recorded on the camera traps.
mm wide black rope to the branch closest to the tent and shaking the branch vigorously for 30 seconds from a distance of six meters. The order in which we tested the different cues was randomly assigned to each group.

The bats’ responses, as well any social or echolocation calls, were recorded with video cameras (Sony Handycam, Model HDR-XR160) and with Avisoft condenser microphones (CM16, Avisoft Bioacoustics, Berlin, Germany) through Avisoft’s UltraSoundGate 416 or 116 H onto a laptop computer running Avisoft-RECORDER software. To measure the response to the different cues, we synchronized video and acoustic files. Movement of the bats’ ears was measured in seconds, number of echolocation or social calls were counted, and any additional behavior such as leaving the roost or opening their eyes were recorded. The same observer conducted all measurements in the synchronized video/acoustic files.

(f) Data analysis: statistics

To test the effects of habitat (mature versus secondary forest) on the number of responses of *D. watsoni* to the different stimuli, we performed an ANOVA analysis on the responses to different cues and whether these responses varied by location. The compound stimuli of squirrel monkey social calls combined with the physical stimuli of branches moving in the mature forest, was excluded from the model analysis since these variables were not replicated in the secondary forest. In the secondary forest, these stimuli were tested separately and were therefore also excluded from the model analysis.
We also performed an ANOVA analysis on the responses to different cues within each forest type, incorporating the cues excluded in the analyses comparing the responses between location. If significant, Tukey HSD pairwise comparison at $\alpha = 0.5$ was used on each analysis to identify the difference in response between the tested cues. Statistical analyses were done using R statistical language and software, version 3.4.3 (R Core Team 2017).

**Results**

(a) *Animal-roost interactions*

The five camera traps recorded 1780 videos of 60 seconds each over a period of 12 months, for a total of 1800 camera days. These included 1138 (63.93%) videos of mammals, 232 (13.03%) of birds, and 19 (1.07%) of reptiles walking under or next to the tents (Table 1). No animals were seen in 377 (21.2%) of the videos. We identified 99.2% of the animals in the videos to 20 species of mammals and birds (Table 1) and categorized the remaining 14 videos as containing unknown species. The Central American agouti, *Dasyprocta punctata*, was the most common species comprising 53.31% (748 videos), followed by the nine-banded armadillo, *Dasypus novemcinctus*, comprising 10.41% (146 videos). *D. punctata*, *Procyon lotor*, *Saimiri oerstedii*, *Tinamus major* and *Crax rubra* were recorded walking under the tents in small groups of up to five individuals, while *Pecari tajacu* and *Nasua narica* were often seen in groups of up to 20 individuals.

Our camera traps show that many species of birds and mammals walked under or next to the tents. From the approximate 30 hours of video clips, we estimate a daily average of 0.92 animals,
or almost one animal or group of animals walking under or next to a tent on a daily basis. We consider this passing rate high, because cameras were placed to observe randomly selected tents avoiding trails where animal traffic could be high, and each tent was recorded only once over a period of a month.

Central American squirrel monkeys, *S. oerstedii*, were recorded in three different video clips from two different roosts. In each case, the squirrel monkeys interacted with the host plants where the roosts were located. One of the videos shows a squirrel monkey below the tent, visually inspecting the roost, then holding the leaf and scanning it. The squirrel monkey then jumped on top of the roost, bringing it down. The recorded videos do not show any squirrel monkey consuming a bat or bats flying out of the roost, so we cannot confirm the presence of bats in the tents. The squirrel monkey was the only known predator recorded interacting directly with the roost. We captured on video other potential predators walking around the tent (Table 1). However, the videos do not show any indication of the animals detecting the roost.

The Central American agouti, *D. punctata*, was recorded on 18 occasions stepping up on two legs and checking young plants of Araceae where the tents were located, apparently searching for infructescence (group of fruit arranged on a stem). If an infructescence was available, they would bite the petiole to gain access to it. *D. punctata* is an herbivore and may not represent any threat to the bats directly, other than altering the bats’ sleep and vigilance behavior.
Table 1. Species list and number of videos recorded by cameras traps at *D. watsoni* roosts.

<table>
<thead>
<tr>
<th>Species</th>
<th>Common name</th>
<th>Diet</th>
<th>Number of videos</th>
<th>% of videos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammals</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Dasyprocta punctata</em></td>
<td>Central American agouti</td>
<td>Hervivore</td>
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<td>53.31</td>
</tr>
<tr>
<td><em>Dasypus novemcinctus</em></td>
<td>Nine-banded armadillo</td>
<td>Insectivore</td>
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<td>10.41</td>
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<td>Paca</td>
<td>Hervivore</td>
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<td>3.56</td>
</tr>
<tr>
<td><em>Pecari tajacu</em></td>
<td>Collared peccary</td>
<td>Hervivore</td>
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<td>2.14</td>
</tr>
<tr>
<td><em>Tamandua mexicana</em></td>
<td>Northern tamandua</td>
<td>Insectivore</td>
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<td>2.07</td>
</tr>
<tr>
<td><em>Nasua narica</em></td>
<td>White-nosed coati</td>
<td>Omnivore</td>
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<td>1.57</td>
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<td>Hervivore</td>
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<td>1.14</td>
</tr>
<tr>
<td>Small rodents</td>
<td></td>
<td></td>
<td>16</td>
<td>1.14</td>
</tr>
<tr>
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<td>Carnivore</td>
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<td>1</td>
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<td>Central American squirrel monkey</td>
<td>Omnivore</td>
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<td><em>Philander Opossum</em></td>
<td>Gray four-eyed opossum</td>
<td>Omnivore</td>
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<td>0.14</td>
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<tr>
<td><em>Leopardus tigrinus</em></td>
<td>Oncilla</td>
<td>Carnivore</td>
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<td>0.07</td>
</tr>
<tr>
<td><em>Marmosa mexicana</em></td>
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<td>Insectivore</td>
<td>1</td>
<td>0.07</td>
</tr>
<tr>
<td><em>Galictis vittata</em></td>
<td>Greater grison</td>
<td>Carnivore</td>
<td>1</td>
<td>0.07</td>
</tr>
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<td></td>
</tr>
<tr>
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<td>Pigeons</td>
<td>Seeds/fruits</td>
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<td>Great curassow</td>
<td>Omnivore</td>
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<td>0.36</td>
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<tr>
<td>Birds</td>
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<td></td>
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<td>Lizards</td>
<td>Insectivore</td>
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<td></td>
<td></td>
<td>1403</td>
<td></td>
</tr>
</tbody>
</table>
(b) Roost behavior

We recorded the diurnal activity of four groups of *D. watsoni* from ~8 AM to 5 PM, or until our observations were interrupted by rain, for an average of 5 hours and 47 minutes of video per group. Overall, bats were motionless for more than ~96% of the time. Non-movement of bats during the day at the roost can be used as an indicator of sleep behavior (Dement and Kleitman 1957). We observed a polyphasic sleep in the groups, with sleep episodes interrupted by brief periods of wakefulness. During wakeful periods that comprised ~4% of the diurnal behavior at the roost, bats opened their eyes, groomed and stretched. These behaviors ranged from 12 seconds to 7 minutes in duration. During these periods of wakefulness, we did not detect any echolocation or social calls.

(c) Behavioral response to different cues

We tested behavioral responses to sound and physical cues in a total of 24 groups of bats in mature and secondary forests, although the behavioral responses of only twenty groups, ten groups per habitat type, were analyzed. Of the four groups excluded, we stopped one of the experiments due to heavy rain. In the other three groups, the bats flew out of the roost when we tested the physical cue of branches moving next to the tent, so it was not possible to test all the cues.

We documented ear movement in response to all the different cues tested and this movement was the only behavioral response that occurred during all the trials. The ear movement response was used for all statistical analysis, and we will refer to it from here on as the bats’ response. During
the playback and physical cue experiments, we did not record any echolocation or social calls under any of the stimuli presented to the bats. However, we recorded echolocation calls as the bats flew out of the roost in response to the physical cues of branch movement in three of the trials excluded from the analysis.

We analyzed the behavioral response to the different cues between mature and secondary forest, finding no significant difference between them (F(1,8)=0.4697, P=0.876). Based on this result, we combined the behavioral response of both forest types into a single model. Using an ANOVA analysis we found a significant difference in the bats’ response to the different cues (F(1,8)=25.0013, P<0.0001). Post hoc analyses were conducted giving the statistically significant omnibus ANOVA F test. Specifically, Tukey HSD tests were conducted on all possible pairwise contrasts (Figure 5).

The vocalization playback cues of the three owl species elicited the lowest response by bats (Figure 5), with no significant difference in the bat responses between the different owl species. However, we observed high variation in the response to crested owls in the mature forest. Our experiment also shows how the bats respond to a novel sound, in this case white noise, which was used as a control. All the bats in the study responded to this cue for a few seconds, then ignored the acoustic stimuli.

The bats showed non-significant response to both echolocation and distress calls of conspecifics (t(34.799)= -1.4633, p=0.1523), although distress calls had a slightly higher response (Figure 5).
**Figure 5.** Number of responses by *D. watsoni* to different cues. The cues presented were Owl (a, b, and c) = *C. nigrolineata, P. perspicillata, and L. cristata* respectively, V.s = *V. spectrum* distress (d) call and echolocation (e) call, WN = white noise, D.w = *D. watsoni* distress (d) call and echolocation (e) call, Twigs = twigs breaking to simulate an animal approaching, Saimiri = *S. oerstedii* monkey social calls, Branches = branches movement next to the tent, S/B = compound stimuli of *S. oerstedii* social calls and movement of branches. Letters over the boxplot represent grouping by the Tukey HSD test.
We obtained opposite responses, with a significant difference, during the playback of echolocation and distress cues from the known predatory bat, *V. spectrum* (*t*(21.695)=8.4961, *p*=0.0001), with a higher response of the tent-making bat to the echolocation calls than to the distress call (Figure 5).

Bats responded the most to the physical cues of branches moving, the auditory cues of breaking twigs, and *V. spectrum* echolocation calls (Figure 5). In response to these cues, we documented that *D. watsoni* not only moved their ears but also opened their eyes and moved their heads around. While we monitored the roosting behavior before the trials, bats only opened their eyes during the short periods of wakefulness for grooming and other social behaviors. We did not observe bats opening their eyes with any of the other cues.

During the first trials in the mature forest, we compounded *S. oerstedii* social calls and movement of branches to have true-to-life stimuli. We observed the highest response with this stimulus combination, and bats stayed alert after we ceased the trial (Figure 5). To understand which element of this stimulus influenced the bats to respond, the two components were tested separately in the secondary forest. The movement of branches next to the roost had the same high response as the compound stimuli. However, the social call of *S. oerstedii* elicited a much lower response, comparable to the bats’ response to the owls’ calls (Figure 5).
Discussion

Our finding of squirrel monkeys interacting with roosts support the observations of Boinski & Timm (1985), who report similar behavior by this species and cite evidence that the monkeys recognize the tents and actively search them for prey. We expected the bats to physically react to the sound of a known predator, but when physical and auditory cues were separated bats responded significantly more to the physical cue (Figure 5). The movement of branches seems to be a more important trigger of the bats’ response, since D. watsoni had the highest response when this cue was tested alone. The physical movement of branches may be an important cue to alert bats of any animal or predator approaching over the roost, since they cannot see above the roost.

The high frequency of animals walking by the tents and the disturbance of the Central American agouti suggest that the breaking twigs cue may have an important effect on the bats' vigilance and sleep behaviors. Tents offer bats a panoramic view of the understory, allowing them to some assessment of predation risks through visual cues. However, when animals, such as squirrel monkeys, approach the tent from above, bats’ ability to assess predation risk is probably more limited. The architectural shape of tents, which vary based on size and shape of the leaf (Kunz and McCracken 1996), limit the field of view for the bats, thus limiting their ability to correctly assess predation risk of animals approaching from out of view. The only cue that resulted in bats flying out of the roost during trials was the movement of branches, therefore suggesting bats are more cautious when an animal is approaching but they cannot assess predation risk visually and, as a consequence, leave the roost.
Our results suggest that owl’s vocalizations may not impose an imminent predation risk at the roost during the day or may indicate that the bats do not recognize the owl calls themselves. Breviglieri et al. (2013) found similar results with the acoustic cues of the barn owl (*Tyto alba*) on foraging behavior activity in frugivorous bats. Bats only display antipredator behaviors in the presence of the visual stimuli of a stuffed owl. Even when owls are quiet during hunting, the use of social calls to attract mates and establish and defend territories can be an indirect cue of their presence in the surroundings. The presence of predators, such as owls, in the environment influences the time that bats leave the roost (Petrželková and Zukal 2001), especially in insectivorous bats that are restrained by the pre-sunset peak in insect abundance (Racey and Swift 1985, Rydell et al. 1996). Since frugivorous bats are not restricted to any daily peak of food abundance, the calls of owls could influence their departure time without missing foraging opportunities for the bats. However, this needs to be explored further by testing these acoustic cues during sunset to coincide with roost departure.

The social calls of male *D. watsoni* are emitted exclusively within 60 minutes of sunrise, suggesting that these calls may play a role in male recruitment of females and the establishment of territory boundaries between males in nearby roosts (Gillam et al. (2013). Our data suggest that even when conspecifics are echolocating or emitting distress calls during the day, *D. watsoni* acknowledge the calls by moving their ears but do not emit any acoustic cue which may reveal the location of the roost.

Like all the Neotropical bats, the predatory bat *V. spectrum* is a nocturnal species and should not be risk for roosting bats during the day. However, our results show showed a high response of *D.*
*Dermanura watsoni* to the echolocation calls, but ignored the distress call of *V. spectrum*. Distress calls attract both conspecifics and heterospecific bats, and this behavior is well documented within the Phyllostomidae family (Ryan et al. 1985, Chaverri et al. 2018), to which both these species belong. *Dermanura watsoni* can discern between social and echolocation calls of both conspecific and heterospecific species, ignoring the distress calls of the predator that do not represent a threat, but showing a high response to the echolocation calls of *V. spectrum* which may represent an imminent threat for the tent-making bats.

We hypothesized that because hearing is highly developed in bats, acoustic cues will provide primary information for assessing predation risk and evaluating when to leave the roost. However, our data show a more complex system where tent-making bats used acoustic cues as the first step in the predation risk assessment. As the risk increases, bats will transition to a wakefulness state and use visual cues to have a more reliable evaluation of the risk. This two-step system may be fundamental to reduce the impact on vigilance and sleep behaviors for species in ephemeral exposed roosts due to the relatively high number of animals moving around the roost.

In conclusion, bats use a two-step system to assess predation risk; acoustic cues are the primary source to evaluate predation risks and, as the risk increases, bats transition to also use visual cues for a more reliable assessment of threats. *Dermanura watsoni* can discriminate social and echolocation calls of both conspecific and heterospecific species. Conspecific identification plays a fundamental role on different social behaviors, such as, location of group members, mating system, and foraging activities. Identification of heterospecific may allow bats to find
new resources, defend resources and identify threats to display an accurate antipredator behavior. Bats use a series of antipredator behaviors, and the behavioral responses were similar across habitats in our study. As an apparent anti-predatory strategy, bats in the roost do not respond or use any call that may reveal their location to potential predators. The use of acoustic cues is particularly beneficial for tent-making bats because these cues allow them to assess predation risk even when threat is not in close proximity to the roost. Additionally, tents provide a panoramic view of the understory, offering bats a visual vantage point to assess predation risk when animals are approaching (Figure 4). However, the physical structure of tents imposes limitations when animals approach above the roost, forcing bats to be more cautious when relying only on acoustic cues.
References


My consistent use of “we” throughout this chapter is in reference to my co-authors and myself. I was the primary contributor to this work, which involved the following tasks: (1) development of project design and all data collection, (2) measurement of acoustic data and statistical analysis, (3) gathering and interpretation of the relevant literature, and (4) all of the writing.

Abstract

Antipredator defenses often rely on perception and discrimination of cues from predators, and alteration of behavior by potential prey. Characteristics of acoustic signals allow eavesdropping on calls of predators, permitting listeners to gauge predation risk by assessing the location and identity of the signaler. We tested the ability of bats that are preyed upon by other bats to discriminate between echolocation calls of predators and non-predators, and the impact of risk reduction strategies on communication and sociality. Bats distinguished between echolocation calls of predators and non-predators, recognizing predator calls with high accuracy. However, bats were more cautious when the structure of non-predator calls was similar to predator calls. In the presence of predator calls, bats ceased social communication, impacting sociality and disrupting group cohesion.
Predation is a major force in nature, shaping a suite of traits to prevent a premature death. Effective antipredator defenses in animals often rely on detection and discrimination of cues from predators versus non-predators that affect prey behavior (Caro 2005). Acoustic cues, in particular, may be valuable for assessing predation risk, as they are easy to pinpoint spatially and temporally, and may readily contain information of species identity and intention that potential prey may use to gauge risk (Rydell et al. 1997). Acoustic signals are fundamental to a diversity of organisms, most notably bats, being used for navigation, foraging, and social interactions (Wilkinson and Wenrick Boughman 1998, Kunz and Fenton 2005). Acoustic signals also have the particularity of being public, and conspecifics are known to eavesdrop on the feeding activity of other individuals (Dechmann et al. 2009). Because some bats are preyed upon by other bats, the ability to eavesdrop on the echolocation calls of bat predators and distinguish them from those of non-predators could be important to avoid predation. However, as demonstrated in other taxa (Caro 2005), anti-predator defenses that disrupt the signaling behavior of prey also may have critical implications for communication and sociality.

Spix’s disk-winged bat (*Thyroptera tricolor*) is a small (4 g), group-living, neotropical insectivorous species that uses the highly ephemeral rolled leaves of *Heliconia* plants as roost sites. These bats rely on a combination of acoustic signals to find occupied roosts and maintain group cohesion; emitting “inquiry calls” during flight, which elicit “response calls” from individuals in occupied roosts (See appendix). We test whether *T. tricolor* eavesdrops on the echolocation calls of other bats, and if individuals gauge predation risk by discriminating among the calls of bats that are predators versus calls of non-predators. We also investigate if *T. tricolor* ceases the emission of response calls when predator calls are present to avoid being detected by
predators. Finally, we investigate the impact of the disruption of inquiry-response calling on social group cohesion.

In playback experiments, we placed an individual *T. tricolor* inside a transparent plastic tube resembling furled *Heliconia* leaves (See appendix) in a soundproof and anechoic chamber (height 0.61 m, width 0.31 m, length 0.51 m). The playbacks were broadcast with a speaker and the bats’ response calls were recorded with an ultrasound microphone (Figure 6A) (See appendix). We performed playback trials of 5.5 minutes per bat, presenting predator echolocation calls from the spectral bat (*Vampyrum spectrum*) and non-predator echolocation calls from a sympatric frugivorous bat (*Uroderma bilobatum*), a sympatric insectivorous bat (*Rhogeessa io*) and a non-sympatric insectivorous bat (*Molossops temminckii*) as a novel control (See appendix). Additionally, we used the inquiry – response social call system of *T. tricolor* (Chaverri et al. 2010) during the trials to induce responses from the bats being tested (See appendix). During the first part of the trial, we used playback sequences of 90s of conspecific inquiry calls, followed by 30s of overlapping conspecific inquiry calls and echolocation calls of the predator, closing with 30s of conspecific inquiry calls. After 30s of silence, the first part was repeated, only changing the echolocation call of the predator for one of the non-predator calls. We alternated the order of predator versus non-predator calls during the trials, as well as altering the different species of non-predator calls. We counted the number of responses during the 30s of inquiry + predator or inquiry + non-predator calls, 30s seconds before, and 30s after (Figure 7).
**Figure 6.** Experimental setup. (A) Schematic of the sound-proof chamber. Bats were placed inside a transparent plastic tube resembling furled *Heliconia* leaves. The microphone (white) and speaker (black) were located directly on top of the artificial leaf. (B) Schematic of flight cage. Circles represent furled *Heliconia* leaves. Four microphones were located outside of the flight cage. A random individual bat was placed inside of a furled *Heliconia* leaf (gray circle) next to a microphone to record whether or not this bat responds to the inquiry calls of the group members.
**Figure 7.** Timeline of the playback experiments. The time in seconds below the horizontal line represents the length of playback sequences. The type of playback sequence is given above the timeline. Inq = inquiry call, Inq + Echo = overlap of inquiry call and echolocation call of either
Of 117 bats tested from 33 different groups, 38 individuals responded during the trials and, in every group tested, at least one member responded to the trial. We observed significant differences in the number of responses before, during, and after the predator trials (linear mixed effect model, $P < 0.001$), showing that bats stopped responding when presented with predator cues (Figure 8A). Also, we observed a difference in the number of response calls before, during, and after non-predator trials (linear mixed effect analysis, $P < 0.009$). However, we observed a higher variability in individuals’ responses to the inquiry + non-predator calls than in response to predator calls (Figure 8B). To understand the variability on the individual response to non-predator calls, we conducted a k-mean clustering analysis of the echolocation calls used during the experiment and compared the results to the number of responses from the bats. The number of responses of *T. tricolor* decreased as similarity of the non-predator call components to the predator calls increased (Figure 9). The cluster analysis shows a high similarity between the predator and the frugivorous bat (Table 2, appendix), which we assume is a consequence of these two species being within the same family (Phyllostomidae) (Kalko 2004, Weinbeer and Kalko 2007), whereas the other species pairs are more distantly related. We used a Signal Detection Theory approach to evaluate the accuracy (proportion of trials garnering a correct response based on our prediction) of the bat responses between each of the non-predator and predator playbacks (See appendix). Bats had the highest accuracy (92.1%) when the predator playback was presented. Insectivorous and novel species control trials had a lower accuracy (75% and 78.6%, respectively). However, the frugivorous calls presented the lowest accuracy (66.7%) response of all. We further tested whether the order of presentation of playback trials ( predator/non-predator
Figure 8. Number of responses to the playback experiment. Comparison of the number of responses before Inq + Echo, during Inq + Echo, and after Inq + Echo (see Figure 7). (A) Response to predator calls. *** $P<0.001$ (B) Response to non-predator calls.
**Figure 9.** Cluster analysis of echolocation call features clustered by playback type and the number of responses to playbacks. The symbols represent the results of the cluster analysis and are circled by a unique color that matches the boxplots. Boxplots represent the number of responses of *T. tricolor* sorted by playback type.
or non-predator/predator) affected bats’ responses (See appendix). We found that the order of presentation affected the number of responses in trials with frugivores (generalized linear model, p=0.018), but not in the other trials. In the experiments where playback of the frugivorous calls was presented first, the number of responses was lower (mean=2.286) than when the frugivorous calls were presented after the predator cues (mean = 23).

To understand the possible effect of social call inhibition by predator acoustic cues, we performed experiments to test if a lack of response calls affected group cohesion. We used a flight cage (9x4x3 m) with six rolled leaves inside the cage (Figure 6B), and four ultrasonic microphones placed outside the cage to record the social calls emitted from bats in the leaves (See appendix). During the experiments, a random individual of each group was left inside one of the rolled leaves. After a few minutes, the rest of the group was released into the cage. We monitored the individual inside the rolled leaf to see if it responded to the inquiry calls of the other bats. We also documented which leaves the rest of the group members chose as a roost (See appendix). In 12 out of the 25 groups we tested, the individual in the leaf successfully recruited group mates to the roost by responding to the inquiry calls from the flying bats. In the remaining 13 cases in which the bat in the leaf did not respond to the social calls of the other bats, the group members roosted in a different leaf. In three of the latter cases, the group further divided into two additional subgroups when no social calls were emitted by the bat in the roost. Our analysis shows a significant effect of the response to social calls on recruiting roost mates to a single roost (Wilcoxon Rank test, W= 156 p<0.001).
There is much evidence that bats recognize and respond to conspecific echolocation calls (Barclay 1982, Balcombe and Fenton 1988). Our results demonstrate that bats can distinguish interspecific echolocation calls, specifically discerning between predators and non-predators. Echolocation calls are used primarily for locating prey and avoiding obstacles, and have different functions, containing less information than communication calls (Beecher 1989, Barclay 1999, Finger et al. 2017). Similar echolocation call structure usually indicates ecological or phylogenetic similarity (Li et al. 2014), and distinguishing between the similarly structured calls of a frugivore and a predator suggests sophisticated discriminatory abilities. This ability is likely essential for species such as *T. tricolor*, that rely on these cues for predator recognition while roosting, thus avoiding behaviors that may reveal an individual’s location while recruiting group members.

The decision-making process of animals during ecologically relevant tasks, such as predator detection, has great biological value, requiring high accuracy to avoid a mortal cost. However, there is a trade-off between speed and accuracy in decision-making (Chittka et al. 2009). Bat echolocation calls are short in duration (milliseconds), requiring fast decision-making processes for accurate identification. Our data show that *T. tricolor* seems capable of high-speed and accurate decision-making by ceasing the emission of response calls as soon as predator cues are detected. However, we observed a decrease in the accuracy of decision making when non-predator calls where more similar to the predator calls. The calls from the frugivorous bat had the lowest accuracy response when they were presented first during the trials, showing difficulty for the individuals to discriminate between two similar calls. Nevertheless, when the frugivorous calls were played after the predator calls, the bat accurately discriminated between them. This
change in the behavioral response could be explained by bats having a predator recognition template early on for comparison (Blumstein et al. 2004). Finally, we observed no delay in T. tricolor’s response to social calls after the predator calls ceased. This behavior can be due to the ephemeral persistence of acoustic signals in space and time, and the speed with which bats fly through an area. Navigation with echolocation requires the constant production of calls, and once the predator calls cease, the receiver may correctly perceive that the signaler is not present in the surrounding area.

Our results suggest that as potential predators are recognized during social signaling, and strategies are adopted to avoid detection, social groups may split. During flight cage experiments, flying bats chose a seemingly random roost and the group was divided when roosting individuals did not respond to the social calls of conspecifics. Thus, we show that predator recognition coupled with strategies to avoid detection may hamper social communication, disturbing group formation and cohesion. If non-signaling roosting bats remain alone, they may need to invest more energy in vigilance and thermoregulation (Krause et al. 2002). Longer search times when flying bats cannot find roosts could also mean greater energetic investments and risk of predation. Ultimately, individuals within less cohesive groups may face lower cooperation rates and reduced fitness (Massen et al. 2010).

This study is the first to show interspecific echolocation recognition in an ecologically relevant setting, allowing bats to make a correct assessment of predation risk and respond accordingly. As predicted by the Signal Detection Theory, roosting bats were more cautious and/or less accurate when deciding to respond to acoustic signals most similar to those of the carnivorous species,
despite the potential costs to social group formation. Our work is the first to demonstrate that these behavioral tactics aimed towards predator avoidance may influence social communication and the maintenance of social groups in bats.
References


Appendix

Materials and Methods

Animals

Groups of 2 to 11, typically 5-6, Thyroptera tricolor roosts inside the developing furled leaves of plants, mainly of the genus Heliconia, Musa, and Calathea which are part of the order Zingiberales (Findley and Wilson 1974) (Figure 10A). Highly specialized, sucker-like structures on their hands and feet, allow T. tricolor bats to hold on to smooth structures like tubular leaves (Wimsatt and Villa 1970, Wilson and Findley 1977) (Figure 10B). Furled leaves are a highly ephemeral roost, remaining within the preferred size for bats between 8 to 16 h and forcing individuals to locate a new roost every day (Vonhof and Fenton 2004). Despite frequent roost switching, T. tricolor maintains a very stable composition of social groups with high relatedness (Chaverri 2010, Buchalski et al. 2014). This species maintains its highly cohesive social aggregation by using social calls (Chaverri et al. 2012), which helps them to recruit group members to the roost. Two sets of calls are used: flying individuals signal with an “inquiry call,” and individuals in roosts signal with a “response call” (Figure 11) (Chaverri et al. 2010). Finally, tubular leaves distort the signal fidelity of the social calls, and the individuals inside the leaf are unable to recognize inquiry calls as being specifically from group members (Gillam and Chaverri 2012). As a consequence, bats in the roost respond to any inquiry call detected, while the flying bats are able to discriminate the response signals from non-group and group member (Chaverri and Gillam 2013). For more information on T. tricolor see Chaverri and Gillam (2016).
Figure 10. Spix’s disk-winged bat (*Thyroptera tricolor*). (A) Top inside view of the bats inside a furled leaf of *Heliconia* plant. (B) Ventral view of *T. tricolor* with suction discs visible on thumbs and feet. Photos: Sébastien J. Puechmaille (A), Melquisedec Gamba-Rios (B)
Figure 11. Social call system of *T. tricolor* for recruiting individuals to the roost. (A) Inquiry call signal emitted by flying bats. (B) Response call emitted by a roosting bat after an inquiry call was received.
We caught 117 Spix’s disk-winged bats from 33 different groups at Hacienda Barú National Wildlife Refuge, located on the South Pacific Coast of Costa Rica. In the early morning, we searched for bats and returned to the field station’s laboratory after capturing two to three new groups. For the duration of the experiment, each group was kept together in a cloth bag in a separate holding area outside of the laboratory where the experiment was conducted. After the experiment concluded all individuals were fed with mealworms (*Tenebrio molitor*) and given water. All bats were released before sunset on the same day and in the same location where they were captured. All the experiments were carried out under the research permits of the Costa Rican authorities (Ministry of Environment and Energy) Permit ACOPAC-INV-021-16 and The University of Tennessee Institutional Animal Care and Use Committee (IACUC 2026-0514) and were consistent with the guidelines issued by the American Society of Mammalogists (Sikes et al. 2016).

**Experiment setup**

*Playback experiment*

We built a soundproof chamber (height 0.61 m, width 0.31 m, large 0.51 m) with glass walls. The inside walls were covered with a felt-like, sound-dampening foam (thickness 5 cm), and the door of the chamber was made of transparent acrylic to observe the individual.

- An Avisoft condenser microphone (CM16, Avisoft Bioacoustics, Berlin, Germany) connected to and Avisoft’s UltraSoundGate 416 or 116 and a laptop computer running Avisoft-Recorder Software was placed on the top center inside the chamber facing down to record the call responses. Additionally, a broadband loudspeaker (Ultrasonic
Omnidirectional Dynamic Speaker Vifa, no. 60108, Avisoft Bioacoustics, Berlin, Germany) was connected to an Avisoft’s UltraSoundGate Player and a second laptop computer running SAS Lab Pro was placed on the top left side facing down to emit the playback recordings during the experiment. Bats were placed inside of a transparent plastic tube (height 0.30 m, top diameter 0.09 m, bottom diameter 0.04 m) resembling furled Heliconia leaves inside the chamber. Artificial roost are readily used by *T. tricolor* without affecting their behavior (Chaverri et al. 2012).

For the playback experiment, we used *T. tricolor* inquiry calls that are emitted by individuals flying out of the roost to induce a response call by individuals in the roost (Chaverri and Gillam 2010). Additionally, we used echolocation calls of the sympatric predator and non-predator bats and echolocation calls from the non-sympatric insectivorous bat (*Molossops temminckii*, Molossidae). This species is not present in Central America and the echolocation calls are distinctly different from any other bat in Costa Rica (Figure 12).

*Flight cage experiment*

We used a flight cage (9 m long, 4 m wide and 4 m height) made of shade cloth and located under trees to reduce high temperatures during the experiments. During each day
Figure 12. Playback calls used during the experiments. Echolocation calls of the different species used during the experiments. (A) non-sympatric insectivorous bat (*Molossops temminckii*, Molossidae). (B) sympatric insectivorous bat (*Rhogeessa tumida*, Vespertilionidae). (C) sympatric carnivore bat (*Vampyrum spectrum*, Phillostomidae). (D) sympatric frugivorous bat (*Uroderma bilobatum*).
of the experiment a new set of six rolled leaves were found in the surrounding forest and brought to the station for use during the trials. Four ultrasonic microphones were used outside the cage to record the social calls. During the experiments, a random individual of each group was left inside one of the rolled leaves. After a few minutes, the rest of the group members were released into the flight cage. We monitored the individual inside the rolled leaf to see if it responded to the inquiry calls of the other bats. We also documented which leaves the rest of the group members chose as a roost.

Data analysis of experiment

The audio recordings were analyzed using SAS Lab Pro (Avisoft Bioacoustics, Berlin, Germany). Each of the 5.5 m playback trial recordings were manually labeled in six 30 s long sections. We then counted the number of responses in each of the sections labeled on the audio recordings from the playback trials (Figure 7). We compared the number of responses before, during, and after predator and non-predator trials using a Linear Mixed-Effect Model made with R (version 3.4.3)(Team 2017), with library lme4 (version 1.1-17)(Bates et al. 2014). We analyzed the similarity in the echolocation calls of the four species of bats used in the playback trials with SAS Lab Pro. For each species, we chose 40 calls that had a high signal-to-noise ratio and were not saturated. The following parameters were measured from each call: peak frequency (kHz; frequency at maximum amplitude), minimum frequency (kHz; minimum frequency in the power spectrum), maximum frequency (kHz; maximum frequency in the power spectrum), and wiener entropy (width and uniformity of power spectrum). These parameters were measured at the start, middle and end of each call. We also measured pulse duration (ms; difference between the start and the end of the call) and distance from start to maximum
amplitude (ms) (Parsons and Jones 2000, Russo and Jones 2002, Biscardi et al. 2004). A k-means cluster analysis was used with the parameters measured on each call, and we specified four groups for the analysis in R. Additionally, we estimate the Euclidean distance values between the four centroids in the k-means cluster analysis in R (Table 2).

Signal detection theory approach was used to measure the ability of *T. tricolor* to differentiate between predator and non-predator calls. We applied rating task to the number of responses in each trial section, using an ordinary rating scale of 4 categories (Stanislaw and Todorov 1999). The proportion of correct responses after the analysis was used to estimate the accuracy of the bats to the different echolocation calls.

To test whether the order in which the playback trials (predator/non-predator or non-predator/predator) was presented affected bats’ responses, we compared the order of presentation using a generalized linear model made with R.
Table 2. Euclidean distance values between the echolocation call of the different bat species in the cluster analysis (See Figure 9).

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CHAPTER III
HUMAN-CAUSED DISTURBANCE AND THE EFFECT OF NATURE-BASED TOURISM ON BATS
I am the primary contributor to this work, which involved the following tasks: (1) gathering and interpretation of the relevant literature, and (2) all of the writing.

Abstract

Nature-based tourism is a growing industry worldwide, with over 8 billion visitors annually to protected areas. This industry generates valuable financial resources, and increasing pressure on protected areas and wildlife as managers try to balance tourism with ecological conservation. Tourists and the disturbances caused by tourists can be perceived by animals as predators or predation risk. These perceived threats can affect fundamental life history processes and behaviors, ultimately decreasing the overall fitness of the animals. The nocturnal habits and the unique life history traits of bats make it difficult to compare the impacts of tourism on bats with such impacts on other taxa. The literature analyzing these impacts on bats is very limited, leaving bats in a perilous condition without a baseline to draw proper conservation initiatives. Here, we review the available information of the impacts of nature-based tourism on bats and enlighten the need of research on areas addressing topics such as behavior, ecology, and physiology, which are fundamental to understanding the unknown consequences of the growing industry on bats.
Tourism has become a major industry, that continues to grow and expand. The impacts and mitigation of this industry have been the focus of many studies over different taxonomic groups (Griffiths and Schaik 1993), such as mammals (Cassirer et al. 1992), birds (Rodgers Jr and Smith 1995, Ellenberg et al. 2006), and reptiles (Amo et al. 2006). Also, most of this body of work focuses on diurnal animals that experience frequent interaction with humans, leaving nocturnal animals, such as bats, lagging in the understanding of the impacts of tourism. The unique combination of life history traits in bats makes it difficult to draw parallels to the impacts of tourism with other taxa. The available information on bats shows that both foliage and cave roosting bats can be disturbed by tourism in natural areas (Mann et al. 2002, van der Aa et al. 2006). Roost requirements have fundamental implications in the behavior and ecology of bats. Human-induced disturbance through tourism could represent a major threat to those bat species restricted to caves or foliage roosts. Here we highlight the need for more studies understanding the behavioral, ecological, and physiological consequences of tourism in bats. Specifically, studies on the impacts of tourism on roost and habitat selection, vigilance, sleep and social behaviors are urgently needed, along with studies addressing the physiological consequences of stress and sleep disruption. These studies are vital to managers of protected areas for the design of strategies to minimize and mitigate the impact of tourism on bats worldwide, in order to balance tourism and bat conservation.

Bats are the second most diverse group of mammals, with almost 1400 species worldwide (Simmons 2005). Despite the diverse ecological roles and well documented environmental services this group of mammals provide (Boyles et al. 2011, Kunz et al. 2011, Ghanem and Voigt 2012), the impact of human activities on bats has not been well studied. This lack of
research is mainly due to the nocturnality of bats limiting their contact with humans when bats are active. Nature-based tourism can induce indirect and direct impacts on bats communities. Visitors to natural areas can trigger changes in vegetation cover affecting the availability of food resources for bats and bats’ prey. Furthermore, tourism can also introduce and spread exotic diseases, increasing the mortality of bat populations. Direct impacts of tourism affect bats’ behaviors, such as habitat and roost selection, vigilance, social behaviors, and key life history moments such as pregnancy, lactation and weaning. Additionally, when tourists disturb bats during hibernation or sleep cycles, they can cause physiological impacts on the bats, increasing stress levels and affecting fat reserves. These direct and indirect effects can increase energetic costs and reduce fitness of bats.

Visitors using protected areas can induce abiotic and biotic ecological impacts (Hill and Pickering 2006). Hikers can provoke soil compaction and, as a consequence, increase soil loss and erosion, which may change the physico-chemical properties of the soils (Arocena et al. 2006, Kissling et al. 2009, Ballantyne and Pickering 2015). Additionally, the activities of visitors can result in the reduction of vegetation cover and the introduction and dispersal of weeds; these effects can lead to changes in plant communities, with potentially cascading effects. Changes in plant production and diversity could, consequently, affect bats through direct loss of food for nectarivores and frugivores and indirect loss of prey items for insectivores as changes in plant communities affect insect abundance and diversity (Vilà et al. 2011, Monz et al. 2013, Ansong and Pickering 2014, Ballantyne and Pickering 2015).
Tourism can also introduce or spread exotic diseases, with documented disease transmission from humans to other species such as primates and other mammals, increasing mortality in wildlife communities (Wallis and Lee 1999, Goldberg et al. 2007). For example, tourists visiting Rwanda in the late 1980’s infected mountain gorillas with human pathogens (Ferber 2000). More recently, since 2006 the cold-tolerant fungus *Pseudogymnoascus destructans*, known as white-nose syndrome, has killed millions of bats across much of the United States (U.S.) and Canada, after the pathogen was spread by tourists from Europe visiting caves in New York State (Warnecke et al. 2012).

When animals encounter humans and stimuli from human disturbance, they can perceive them as predators or predation risk (Frid and Dill 2002, Gaynor et al. 2018). Even when human activities are non-lethal, they may affect animal behavior (Preisser et al. 2005, Peckarsky et al. 2008) and animal populations (Ellenberg et al. 2006, Baudains and Lloyd 2007). Visitors to protected areas can disturb wildlife, affecting and changing wild animals’ behaviors (Lusseau 2004, Walker et al. 2006, Steven et al. 2011), such as habitat selection and vigilance. Habitat selection is a trade-off between risk and resource richness. Animals spend less time in areas with both high resources and high predation risk (Lima 1998a). For example, after wolves (*Canis lupus*) were reintroduced in Yellowstone National Park, the resident populations of elk (*Cervus elaphus*) in the Park favor areas of high visitor traffic including roads, (White et al. 2009, Nelson et al. 2012), leading to a human-induced predation refugia for resident elks on roadways and tourist areas (Nelson et al. 2012). However, if no suitable alternative habitats are available animals may not be able to shift habitats under increased predation risk or disturbance stimuli (Gill et al. 2001). As a consequence, animals inhabiting tourism areas may increase energetic costs by
frequently using antipredatory behaviors, including vigilance (Frid and Dill 2002). Vigilance increases the chances of prey avoiding capture, since prey are more vigilant as the perceived risk of predation increases. Perceived risk can increase when disturbance stimuli approach directly, or remain in close proximity, and with increased group size (Lima 1998b). As a consequence, prey reduce time on other activities (Lima and Dill 1990), such as foraging and social activities, and may also decrease body condition due to increased stress (Pérez-Tris et al. 2004).

Human-induced disturbance can also alter short-term behavioral patterns such as flight initiation distance (FID), which is the distance at which an animal begins to flee from an approaching predator (Hediger 1934, Ydenberg and Dill 1986). This distance should increase when predators approach directly, as an indication of detection and intent to capture (Burger and Gochfeld 1981, Cooper Jr 2003). Also, FID should increase with increasing size, number, or speed of the predators (Walther 1969, Dill 1974). Flight initiation distance has been used by wildlife managers to demarcate exclusion zones for visitors, beyond which species are not impacted by the humans (Rodgers Jr and Smith 1995, Fernández-Juricic et al. 2005). However, FID is known for only a few species of bats, such as *Myotis velifer* in the U.S. and *Rousettus madagascariensis* in Madagascar (Mann et al. 2002, Cardiff et al. 2009, Cardiff et al. 2012). Disturbance stimuli can also disrupt sleep patterns in animals, having varying impacts on different species (Kight and Swaddle 2011). Sleep plays a vital role in all animals, providing critical restorative functions (Rechtschaffen 1998), and evidence shows that sleep-deprivation over long periods of time can lead to death (Rechtschaffen and Bergmann 2002). In mammals, daily amounts of sleep vary substantially between species. Mammals show an inverse relationship between body mass and sleep amount (Siegel 2005). Large mammals, such as
elephants, sleep around 3-4 hours a day, while bats sleep 15-20 hours a day (Zhao et al. 2010). The distribution of sleep across the diel (daily) cycle should balance the trade-offs between predation risk, needs for sleep, and time for other activities, such as foraging. The distribution of sleep states may change as the risk of predation increases. Animals might reduce sleeping time, increase arousal frequency, and reallocate sleep to different times of the day in response to increased predation risk (Lima et al. 2005). Sleep disruption by human disturbance may be stronger for nocturnal animals, especially bats that roost in areas exposed to human activities, rather than hidden in structures such as burrows or dens. Human activities, both lethal and nonlethal, influence and change wildlife diel cycle patterns. Worldwide, in areas exposed to human activities, animals shift their patterns and increase nocturnality to avoid encounters with people (Gaynor et al. 2018).

Humans can have a direct impact on bat roosts during the day, when bats should be sleeping. Human hunters have been a real threat to some bat species over evolutionary time, especially for Old World fruit bats on Indian and Pacific Ocean islands (Pierson and Rainey 1992, Mickleburgh et al. 2002, Tsang 2018). Bats are usually hunted at roosting sites, where they aggregate (Jenkins and Racey 2008, Tsang 2018). As examples, villagers hunt multiple species of Madagascar’s bats at the roosts sites, knocking them out of the roost with sticks or swatting them as they emerge (Jenkins and Racey 2008). The intense consumption of flying fox bushmeat in North Sulawesi, Indonesia has led to the extirpation of some bat species in the region, and, as a consequence, bat bushmeat traders encourage local villagers in other regions to hunt bats at their roosts to keep up with the demand (Tsang 2015). A few locals using fishing hooks and ropes could gather tens to hundreds of flying foxes per day, and this hunting strategy may
deplete an entire colony in a single season (Tsang 2018). Australian Pteropodids have been hunted to reduce economic impacts on orchards (Garnett et al. 1999). Today in most of these areas, hunting has been prohibited. However, Pteropodids in the Philippines fly more frequently from tree day roosts in response to visitors and human-induced disturbances (van der Aa et al. 2006). In these species, from the animal’s perspective, disturbance stimuli and true predatory stimuli may be indistinguishable.

The potential effects of tourism on the behavioral response of bats have been measured for the endemic Pteropodid bat (*Rousettus madagascariensis*) in Madagascar’s caves. Behavioral changes were observed in both low- and high- intensity visits to the colonies. The visits to the caves increased flights and alertness of the bats. This was triggered by direct illumination and close proximity of ca. 5-6 meters by visitors to the bats (Cardiff et al. 2012). Additionally, similar experiments were conducted on two maternity colonies of cave myotis (*Myotis velifer*) in Arizona, U.S. (Mann et al. 2002). The researchers manipulated different factors, such as size of the tour group, whether tour groups talked, distance of the group to the bats, light intensity, and color of light. Cave bats were disturbed by the tours in both colonies. Behavioral responses were higher when artificial light was used on tours, and bats had higher vocalizations when white light was used over red light. Flight initiation distance of the bats was around eight meters, and bats increased takeoffs and activity levels when tours passed close to the bats. Tour groups talking also increased flights and activity levels (Mann et al. 2002).

The effect on bats of humans visiting caves has received particular attention since at least half of bat species worldwide rely on caves as roosts (Kunz 1982). In temperate zones, human
disturbance may have significant consequences on key life history moments, such as hibernation, having important physiological impacts and affecting fat reserves in these bat species (Thomas 1995). Maternity colonies in caves are particularly susceptible to human disturbance, leading adults to drop their young and not recover them as they respond to disturbance. As a consequence, caves with high disturbance rates often exhibit a decrease in bat populations. These phenomena have been documented in the cave myotis (*Myotis velifer*), the ghost-faced bat (*Mormoops megalophylla*), and different species in the genus *Pteronotus* (Mann et al. 2002, Petit et al. 2006, Deleva and Chaverri 2018). Curacao Island in the Caribbean observed the complete disappearance of *M. megalophylla* from Grot van Hato Cave after the cave become a tourist attraction (Petit et al. 2006). In Cambodia, the abundance of Buddhist shrines and temples in caves has led to an increase in visitation in recent years, with caves such as Vihear Tuk-Bonn receiving more than nine thousand visitors annually (Lim et al. 2018). This visitation during the Khmer New Year in April coincides with the major peak of birth in two bat species, Horsfields leaf-nosed bat (*Hipposideros larvatus*) and black-bearded tomb bat (*Taphozous melanopogon*) (Lim et al. 2018). The impact of visitor disturbance on the bats populations in these Cambodian caves has not been assessed due to the absence of historical data on bat populations.

The effects of tourism on foliage-roosting bats, such as tent-making bats in the Neotropics, is unknown. Modified leaves as tents can be found occasionally next to trails, where tourist walking and talking could alter sleep and vigilance behaviors of the bats. The behavioral response of the tent-making bat, *Dermanura watsoni*, to acoustic cues of animals or people approaching the roost shows that bats respond to these cues with increasing activity as the presented stimulus increases the perceived risk of predation (Chapter one of this dissertation).
This risk assessment could increase the stress levels on the bats with physiological consequences when it is repeated or prolonged. The flight initiation distance of tent-making bats seems to be very short, as the bats are cryptic under the roosts (Rodríguez-Herrera et al. 2007) and flying out is a last resort strategy. The difficulty of approaching the roosts of *D. watsoni* without the bats leaving their tents is well known to bat researchers. This could be because the roost limits the field of view, restricting the acquisition of information on predation risk. Overestimating risk may have lower fitness consequences than the cost of underestimating the predation risk, which could lead to death (Bouskila and Blumstein 1992).

Despite the different impact visitors in natural areas can have on bats, tourism provides a unique opportunity for environmental education and awareness (Medellin 2003, Ladle et al. 2012), and conservation of bats (Medellin et al. 2017). The U.S. and Canada have more than 125 places to view bats (BCI 2000). Caves, such as Bracken Cave in Texas, U.S., which holds the largest bat colony worldwide with over 10 million individuals, provide the opportunity to increase education and awareness of spectators through guided tours that do not disturb the bats when they are emerging from the cave. Caves with bat colonies in Brazil, Turkey and Australia receive more than 300,000 visitors annually (Lobo and Moretti 2009, Akca et al. 2016, Medellin et al. 2017). Additionally, conservation organizations, such as Lubee Bat Conservation and Bat Conservation International in the U.S., Latin-American Network for Bat Conservation (RELCOM), United Nations Environment Program/EUROBATS, the Southeast Asian Bat Conservation Research Unit (SEABCRU) and the Chiroptera Conservation and Information Network of South Asia (CCINSA), play important roles in each region, providing ways for the
public to enjoy wildlife without disturbance, as well as promoting awareness and conservation of those areas.

When tourism is managed properly, it can promote environmental awareness and protection, as well as generating financial resources (Pennisi et al. 2004). Successful initiatives allow people to view bats emerging from their roost without directly impacting the bats. Carlsbad Caverns National Park in New Mexico, U.S., with over 50,000 annual visitors, and the Congress Avenue Bridge in Austin, Texas, U.S., with more than 140,000 annual visitors, have become major attractions where visitors passively observe large colonies of the Mexican free-tailed bat (Tadarida brasiliensis) emerging from their roosts, generate more than $4,500,000 a year in direct revenue (Bagstad and Wiederholt 2013). Initiatives such as these, could be replicated in other large colonies of bats worldwide, providing both financial and educational benefits.

Regardless of the benefits, tourism can have negative effects on wildlife. There is an increase pressure by tourists looking for opportunities to have a closer encounter with wildlife, including bats. For example, Proboscis bats (Rhynchonycteris naso), which roost on the bark of trees leaning over rivers in the Neotropics, are exposed to several tourist boats approaching them daily (personal observation). The frequent approach by tourists to foliage-roosting bats can disrupt sleep behaviors, increase vigilance, hinder social behavior, and have other physiological impacts on bats. However, for most of the species exposed to nature-based tourism, both behavioral and physiological consequences are unknown.
Tourism has become one of the largest industries worldwide and contributes more than 7 trillion dollars annually to the global economy (Ohlan 2017, W.T.T.C. 2017). At the local level, nature-based tourism provides financial resources to local communities, as well as to protected area agencies, which require funds for conservation efforts (Morrison et al. 2012, Steven et al. 2013). Worldwide, protected areas receive an estimated 8 billion visitors per year (Balmford et al. 2015). This number of visitors is a significant alternative source of fiscal resources for management and conservation of protected areas (Bookbinder et al. 1998, Emerton et al. 2006, Buckley 2009). A constant increase of visitors to natural areas disrupts key life history and natural processes, which threaten bats’ overall fitness and survival, leaving managers in the paradox of needing to balance the ecological impact by visitors with financial support for protected areas.
References


CONCLUSION

Donald Griffin, who first revealed the secret of echolocation in bats in the late 1930’s, referred to bats’ use of sonar as a “magic well” because the more we learn the more surprising discoveries come to light (Griffin 1958). Over the last 80 years, many studies have investigated how eavesdropping on conspecifics plays a vital role in locating potential foraging sites, and this behavior has been observed in different insectivorous species such as *Myotis lucifugus* (Barclay 1982), *Lasiurus borealis* (Balcombe and Fenton 1988), *Rhinopoma microphyllum* (Cvikel et al. 2015), *Tadarida brasiliensis* (Gillam 2007), *Euderma maculatum* (Leonard and Fenton 1984), and in the fishing bats *Noctilio albiventris* (Dechmann et al. 2009), and *N. leporinus* (Übernickel et al. 2013). This behavior may be a benefit for foraging because it expands the search area, increasing the opportunity to find high-quality patches of prey by increasing the maximum detection distance. Individuals using echolocation alone can detect prey from a few meters away, while individuals eavesdropping on conspecifics calls can detect prey at a distance of up to 54 meters (Dechmann et al. 2010). Additionally, echolocation calls exert a selective pressure on the evolution and behavior of their prey (Rubin et al. 2018). However, how bats rely on sounds to adaptively modify their behavior and avoid predation has not previously been investigated.

In this body of work, I showed how bats detect and discriminate predators, which are essential mechanisms for any antipredator strategy. Bat uses auditory cues as the first filter to rate predation risk and, with escalating risk of predation, bats transition to visual cues. Visual and auditory cues coupled together provide a more reliable assessment of any threat. Additionally, bats distinguished between echolocation and social calls of predators and non-predators,
recognizing predator calls with high accuracy. Bats were more cautious when discriminating between structurally similar calls of phylogenetically related species, behaving more cautiously and reducing the accuracy of their decision making.

Here I also describe the predator avoidance behaviors display by the bats, after a predator is detected. Tent-making bats do not produce any echolocation or social calls during the day in their roost. Thomas’s fruit-eating bat (*Dermanura watsoni*) uses social contact calls early in the morning to recruit group members (Gillam et al. 2013). However, *D. watsoni* did not produce any acoustic response to the playbacks of conspecifics, showing a strong antipredator behavior to avoid conveying their location to any possible predator. The Spix’s disk-winged bat (*Thyroptera tricolor*), responded to the contact calls of conspecifics outside the roost. Yet, when predator calls were detected, the individual inside the roost immediately stopped responding to contact calls. This behavior illustrates the importance of eavesdropping on antipredator behaviors.

Antipredator behaviors are intended to avoid or elude predators, but these behaviors may have unintentional implications on intraspecific communication. I showed that in the presence of predator calls, *T. tricolor* stop responding to social signaling of conspecifics, disrupting a key system used to recruit group members of this species into the roost. The results of the experiments show that when individuals did not respond to the social calls of conspecifics, group members flying outside the roost chose other roost options and the group was divided. These results show that behavioral mechanisms aimed toward predator avoidance may have critical implications for sociality.
Group formation and cohesion are fundamental for different mechanisms that enhance overall fitness of the group members. Living in groups brings benefits, such as access to mates, reduced predation (Hill and Dunbar 1998), thermoregulation (Ancel et al. 1997), increased foraging efficiency (Yip et al. 2008), and potential cooperation (Silk et al. 2003). Additionally, sociality reduces the energetic costs of movement, in particular in a gregarious species, such as T. tricolor, which uses highly ephemeral roosts and individuals need to locate new roost sites every night. Cooperation of individuals in a stable group will reduce time and energy cost searching for new roosts, yet in a disrupted group, longer search times could result in greater energetic investment and increased predation risk. As a consequence, individuals may face lower cooperative rates, reducing fitness over the individuals.

The body of work presented in this dissertation clarifies some of the predator-driven behaviors displayed in foliage-roosting bats. Understanding these behaviors could be essential to face humans and human-induced disturbance, which are perceived as predators or predation risk stimuli by bats. This is a growing threat for bat populations in natural environments, which have been overlooked for studies on human-induced threats. Here I discuss how more information is needed to assess fully the influence of tourism on bats, discussing the potential impacts and reviewing the available information. Based on this information, I discuss how tourists disrupt critical life history moments and behaviors, such as sleep, vigilance roost and habitat selection. Ultimately, tourism can decrease individual fitness of wildlife, endangering communities of bats in natural areas.
References


VITA

Melquisedec Gamba-Rios was born in Colombia on February 20, 1980. He completed his bachelor’s degree in 2006 in Ecology and Conservation at the Universidad Latina de Costa Rica, in San Jose, Costa Rica. The following years, he worked as the academic coordinator for Tirimbina Rainforest Center in Sarapiqui, Costa Rica. During those years, Melquisedec coordinated and taught field courses in Tropical Ecology, Tropical Biology, and Conservation for multiple universities in the United States. In 2012, he entered the doctoral program in the Department of Ecology and Evolutionary Biology at the University of Tennessee. M Gamba-Rios has published his work in multiple scientific journals, including Animal Behavior, Ethology, and Journal of Mammalogy.