Acoustically mediated predator detection and risk assessment in the fall field cricket, *Gryllus pennsylvanicus*

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Abstract

Insects are highly acoustic animals that use acoustic sensory systems for a variety of purposes, including detection and evasion of predators. The focus of research on acoustically mediated predator detection in insects has been on detection of bat predators by eavesdropping on echolocation signals. However, insects are prey to an array of non-bat predators that produce acoustic signals and cues that may be used to evade predators. It has been proposed that insects may use such sounds and vibrations for predator detection, but evidence for this is limited. I tested the hypothesis that insects use sounds and vibrations from non-bat predators for predator detection and risk assessment using a locally abundant species of field cricket (*Gryllus pennsylvanicus*) as a model organism. Live recordings of American toads (*Anaxyrus americanus*) and playbacks of pre-recorded sounds from breaking branches and communication signals and flight sounds from insectivorous birds showed that non-bat predators of *Gryllus pennsylvanicus* produce sounds that at least partially overlap with sensitivity of their tympanal organ. Recordings of vibrations produced by live toads and playbacks of pre-recorded bird communication signals, bird flight, and breaking branches showed comparatively less overlap with vibrational sensitivity. Playbacks of bird communication signals and bird flight induced freezing responses in *G. pennsylvanicus*, suggesting that crickets use these sounds to detect and evade predators. This study provides the first evidence that fall field crickets are attending to sounds produced by non-bat predator species to avoid predation.

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Chapter 1: General introduction

Predator detection and risk assessment encompass a suite of behaviours associated with gathering information from the environment to evaluate dangers or potential dangers, including the presence of predators and parasitoids (Blanchard *et al.* 2010, Fischer and Frommen 2019). This benefits animals by allowing them to defend themselves against threats (Ydenberg and Dill 1986, Ellard and Eller 2009). Prey can gather information for risk assessment through stimuli received from threats (e.g., from predators or parasitoids) or non-threats (e.g., from conspecifics or non-threatening heterospecifics) (e.g., Schmidt *et al.* 2008, Goodale *et al.* 2010, Barrera *et al.* 2011). This may include, but certainly is not limited to, scents or sounds produced by predators, wind stimuli generated by predator movement, and alarm calls from conspecifics or heterospecifics (Zimmerman and Kight 2016). Prey gather such information using a number of sensory modalities including those that detect chemical, visual, and mechanical stimuli. In the context of risk assessment, chemical sensing involves the use of olfactory and gustatory receptors to detect chemicals that inform prey of risk (reviewed by Zimmerman and Kight 2016). Chemical stimuli used for predator detection include chemicals left behind by foraging predators, predator kairomones, and alarm pheromones produced by non-threatening species (reviewed by Weiss 2019). Visual sensing for risk assessment involves the use of visual stimuli such as shadows, movement, or looming figures that may indicate the presence of a threat. Such stimuli can be detected by photoreceptors located in the retina of vertebrates and compound eyes and/or ocelli in many invertebrates (reviewed by Inman 2021, Williams 2022). Mechanical sensing refers to the detection of mechanical stimuli such as contact (e.g., touch), wind, or vibrations transmitted through air or solids (i.e., acoustic stimuli).

In the context of risk assessment, animals can detect predators by contact through mechanoreceptors present in the skin (vertebrates) or antennae (invertebrates). Prey may also detect changes in wind due to predator movement through displacement of setae such as trichoid sensillae in invertebrates, including those located on cerci in many species of insect (Dupuy *et al.* 2011). Acoustic stimuli are transmitted as vibrations through elastic media such as air, water, or solids (Figure 1.1) (Yack 2016). Airborne acoustic stimuli generated by predators are transmitted by either the displacement of air molecules (i.e., near-field sound) or through the creation of pressure waves that may travel long distances from the source (i.e., far-field sound) (Yack 2004). The displaced air molecules can be detected by lightweight structures such as the trichoid sensilla on the body surface and/or lightweight antennae that stimulate the Johnston's organ in insects (Yack 2004, Windmill and Jackson 2016). Sound propagated as pressure waves is detected by displacement of the tympanal membrane by sound waves in the tympanal ears of both vertebrates and insects (Yack 2004, Yack and Dawson 2008). Vertebrates and many insects have well-developed senses of hearing that can be used in predator detection and risk assessment (reviewed by Yack *et al.* 2020). My thesis will focus on the use of sounds and vibrations for the detection and assessment of risk in insects.

Acoustic stimuli that animals use for risk assessment can be broadly categorized as either signals or cues (Figure 1.2). Signals are stimuli that have evolved to carry information to an intended receiver, including oneself (e.g., echolocation), to generate a response (Maynard-Smith and Harper 2003, Yack *et al.* 2020). While signals are generally received by their intended receivers, unintended receivers may also

eavesdrop on signals to benefit from the information conveyed. For example, bats and cetaceans produce echolocation signals to communicate with themselves by sending a sound pulse and receiving the echo to navigate their environment and assess the location and characteristics of prey. These echolocation signals can in turn be eavesdropped upon by unintended receivers, including ultrasound sensitive fish preyed upon by cetaceans or flying insects preyed upon by bats (Miller and Surlykke 2001, Wilson and Dill 2002, Yager 2012; Pollack 2015, ter Hofstede and Ratcliffe 2016). Prey may also benefit from eavesdropping on signals produced by non-predators such as alarm calls produced by either conspecifics or heterospecifics. For example, red-breasted nuthatches eavesdrop on and respond to mobbing alarm calls produced by black-capped chickadees (Templeton and Greene 2007). Cues, as opposed to signals, are passive stimuli generated either by a predator or non-predator that have not evolved to alter the behaviour of a recipient. These include incidental sounds generated by movement (Yack *et al.* 2020). Cues used by vertebrate prey in the context of risk assessment include sounds and vibrations generated by predators while the latter are foraging (e.g., flight sounds, rustling grass, footsteps), escape sounds from conspecifics, and escape sounds from heterospecifics. For example, white-browed scrubwren nestlings cease calling in response to playbacks of predators walking through leaf litter (Haff and Magrath 2010), and male Túngara frogs decrease their calling rate in response to playbacks of flight sounds of frog-eating bats (Bernal *et al.* 2007). In vertebrates, detection of both prey and predators is considered to be a primary function of their acoustic sensory system (Fay and Popper 2000). In insects, while the role of hearing in the context of communication and mating has been explored at length

(reviewed by Göpfert and Hennig 2016, Baker *et al.* 2019), how insects use sounds and vibrations in detection of predators and assessment of risk is comparatively underappreciated.

Insects are highly acoustic animals that rely on acoustic stimuli for a variety of functions including species recognition, courtship, territoriality, aggression, and predator detection (Pollack *et al.* 2016). As such, insects have evolved receptors to detect acoustic stimuli in the form of solid-borne vibrations, near-field sound, and far-field sound. Insects use different sensory organs to detect solid-borne vibrations, the best-known being the subgenual organ located in the proximal tibia of most insects (Yack 2004, Turchen *et al.* 2022, Virant-Doberlet *et al.* 2023). In the context of predator detection and risk assessment, some insects are known to detect and use vibrations generated by approaching predators to mediate antipredator responses (e.g., Acheampong and Mitchell 1997; Kiyotake *et al.* 2014; Oberst *et al.* 2017; Gish 2021). Near-field sound is an important but underappreciated source of information for many insects (Yack 2004, Windmill and Jackson 2016). Typically, near-field receptors are sensitive to low-frequency vibrations (under 1000 Hz) from nearby sources (Greenfield 2002, Menda *et al.* 2019). There are two main types of near-field receptors: trichoid sensillae and Johnston's organs. Trichoid sensillae are hair-like cuticular projections found in various locations dependent on insect species (Tautz and Markl 1978). The Johnston's organ is located at the base of the antennae and detects antennal displacement by near-field sound in many insects (Yack 2004). Near-field hearing is not particularly well-studied in the context of risk assessment; however, these receptors are thought to be important for the detection of the wingbeats of approaching aerial insect

predators. For example, the thoracic trichoid sensilla of several species of lepidopterous caterpillars are sensitive to near-field sounds produced by parasitic and predatory wasps or flies (e.g., Markl and Tautz 1975, Tautz and Markl 1978, White *et al.* 1983, Taylor and Yack 2019). Far-field sounds are detected by tympanal ears in insects. Tympanal ears are composed of a tympanal membrane and an associated air sac which allows the membrane to vibrate in response to sound pressures to detect far-field sound (Yack 2004). Tympanal hearing has been shown to be important for insects in a variety of contexts including aggression, territoriality, courtship, and predator detection (Yack 2004, Göpfert and Hennig 2016). In the context of detecting predators, insect tympanal hearing has almost exclusively been investigated in the context of evading attacks from bats by eavesdropping on their echolocation signals (Hoy 1989, Miller and Surlykke 2001, Yager 2012, Pollack 2015, ter Hofstede and Ratcliffe 2016, Yack *et al.* 2020). Many flying insects, including moths, mantids, beetles, lacewings and katydids have evolved ultrasound-sensitive ears for bat detection. Behavioural and neurophysiological studies have shown that such insects respond both neurally and behaviourally when echolocation calls of bat predators are played back (e.g., Fullard 1994, Pollack and Martins 2007, Jacobs *et al.* 2008) However, apart from detecting and evading bat predators, little is known about how insects use tympanal hearing for risk assessment.

It is reasonable to hypothesize, for several reasons, that some insects would use their tympanal hearing to detect non-bat predators. First, many insects that have ears may use them for more than one purpose. There are many species of insects (e.g., crickets, cicadas, katydids) that are thought to have ears dedicated for reproductive

behaviours that could also use hearing to detect predators (Yack *et al.* 2020). For example, several night-flying cricket species use tympanal ears to detect both conspecifics (either mates or rivals) and bat predators (Pollack and Martins 2007, Schöneich *et al.* 2015, Hedwig 2016, Pollack *et al.* 2016). Second, there are several tympanate insects that are capable of hearing, but with no known function to date, and in these cases the proposed function is predator detection (Yack *et al.* 2020). For example, there are many species of butterflies and some grasshoppers that do not communicate with sound, but still possess broadly tuned and sensitive tympanal ears, and these have been proposed to function in risk assessment (e.g., Lane *et al.* 2008, Lucas *et al.* 2009, Lehmann *et al.* 2010, Lehmann 2012, Sun *et al.* 2018). Third, many of the non-bat predators produce sounds, either as signals, or incidental cues (e.g., flight sounds, rustling grass, foraging sounds) (reviewed by Yack *et al.* 2020). Considering these points, it is reasonable to assume that some insects have evolved the ability to eavesdrop on the sounds of their non-bat predators. There is currently indirect evidence for acoustic detection of non-bat predators in insects. For example, the tympanal ears of some moths and butterflies, based on neurophysiological recordings, are capable of detecting rustling sounds of birds landing in bushes, bird flight sounds and bird calls (e.g., Jacobs *et al.* 2008, Fournier *et al.* 2013, Mikhail et al. 2018). There is also evidence that moths respond behaviourally to bird foraging sounds by fleeing (Jacobs *et al.* 2008). In this thesis, I will study the role of hearing in predator detection and risk assessment in a well-studied tympanate insect, the field cricket.

Field crickets (*Gryllus* spp.) have been extensively studied for their acoustic sensory systems, primarily in the context of reproductive behaviour (reviewed by

Schöneich 2020). Males produce both calling and courtship songs through stridulation to advertise to and court receptive females, as well as in territorial and aggressive interactions with other males. The long-range calling songs are used by males to attract females from a distance before turning to quieter courtship songs when in contact with a female (Alexander 1961). Females listen to and use both calling and courtship songs for mate choice, with females generally preferring males that call more frequently and courtship songs with higher chirp rates and longer chirp durations (Hedrick 1986, Wagner 1996, Holzer *et al.* 2003). As such, most field cricket ears are tuned to the frequency and temporal patterns of their communication signals with flight-capable species (e.g. *Gryllus bimaculatus*, *Gryllus texensis*) showing sensitivity to ultrasound as well (e.g., Paton *et al.* 1977, Schildberger *et al*. 1988, Pollack and Martins 2007, Schöneich *et al.* 2015, Hedwig 2016). It is important to note that crickets are prey to a variety of predator species, including insectivorous birds, reptiles, amphibians, and mammals (Hedrick and Kortet 2012). Chapter 2 provides further discussion of cricket predators. These predators likely generate sounds and vibrations, such as those produced by foraging, that crickets may use for risk assessment.

In my thesis, I test the hypothesis that insects use sounds and vibrations generated by natural enemies for risk assessment. I use the fall field cricket (*Gryllus pennsylvanicus*) as a model organism. To test this hypothesis, I generated the following predictions: 1) Natural enemies of *G. pennsylvanicus* produce sounds and vibrations relevant to crickets; 2) Sounds and vibrations produced by natural enemies of *G. pennsylvanicus* will overlap with the sensitivity of cricket acoustic sensory organs; and 3) *Gryllus pennsylvanicus* will respond to sounds produced by natural enemies with

antipredator behaviours. I tested these predictions in two experiments. The first experiment involved recording and characterizing acoustic stimuli produced by a variety of predators of *G. pennsylvanicus* to test the predictions that predators of *G. pennsylvanicus* produce acoustic stimuli, and, that these stimuli overlap with cricket hearing sensitivities (Chapter 2). The second experiment was a sound playback experiment using sounds characterized in the first experiment (Chapter 3). The goal of this experiment was to test the prediction that *G. pennsylvanicus* will exhibit antipredator behaviours (e.g., freezing or fleeing) when presented with sounds produced by their predators.

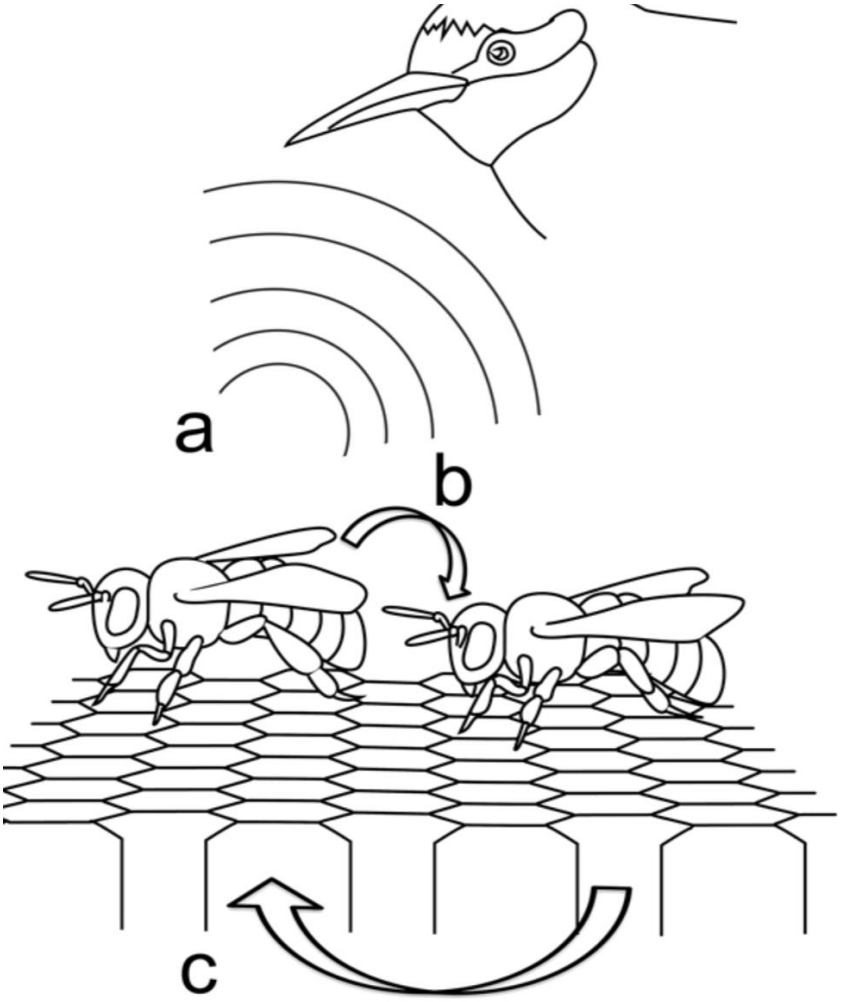

Figure 1.1. Illustration of different types of acoustic signals (i.e. sound and vibration) using the example of a honeybee. **(a)** Hissing sounds that function as antipredator signals are generated by vibrating wings and are detected by the pressure-sensitive ears of a vertebrate predator. This is an example of far-field sound acting as an acoustic signal. **(b)** A dancing forager communicates information about a food source to a recruit through dorsoventral oscillations of its wings. These sounds are detected by the antennae (Johnston's Organ) of the recruit. This is an example of near-field sound being used as an acoustic signal. **(c)**. A recruit can transmit solid-borne signals through the wax comb by pressing its thorax against the substrate and vibrating. These vibrations can be detected by leg receptors (subgenual organs) of the forager. This is an example of solid-borne vibrations acting as an acoustic signal. (Figure reproduced from Yack 2016).

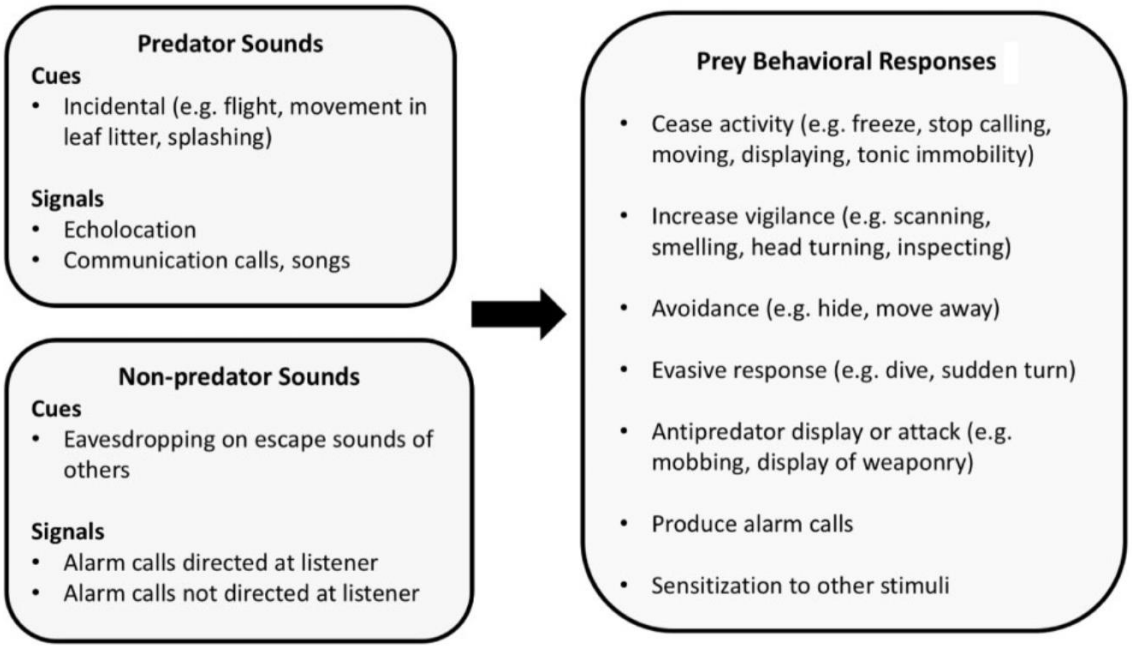

Figure 1.2. Overview of the types of sounds prey use to avoid predation and how they respond to these sounds. (Figure reproduced from Yack *et al.* 2020).

Chapter 2: Characterization of acoustic cues (Experiment 1)

Introduction

Insects have evolved sensitivity to acoustic events (sound or vibrations) multiple times (Yack and Dawson 2008, Hoy and Yack 2009). They rely on sounds and vibrations in a wide range of non-mutually exclusive contexts including those associated with reproduction, aggression, host location, social interactions, and predator detection (Greenfield and Baker 2003, Yack and Dawson 2008, Hoy and Yack 2009). In the context of predator detection and risk assessment, most research has focused on flying insects that detect and avoid ultrasonic signals of echolocating bat predators (Miller and Surlykke 2001, Strauß and Stumpner 2015, Göpfert and Hennig 2016). However, insects face the threat of predation and parasitization by an array of non-bat species, including other invertebrates, birds, mammals, amphibians and reptiles (Yack *et al.* 2020). Do insects use sounds and solid-borne vibrations to detect non-bat predators, as is seen for many vertebrates?

Acoustically-mediated risk assessment in insects comprises the use of sounds (i.e., vibrations transmitted through air) or vibrations (i.e., vibrations transmitted through solids) to assess risk of attack by natural enemies (Yack *et al.* 2020). Sounds and vibrations can be classified as either signals or cues (see Figure 1.2 in Chapter 1). Such signals or cues used in risk assessment by a receiver may arise from the predator itself or a non-predator (see Figure 1.2 in Chapter 1). Signals are broadly defined as stimuli that have evolved to convey a message to a receiver, whether that be another individual or oneself. Predator signals that an insect may use for risk assessment include echolocation signals and communication calls and songs. For example, many species of insects are known to eavesdrop on bat echolocation signals to avoid predation by bat

species and respond behaviourally to them with negative phonotaxis or by freezing (reviewed by Miller and Surlykke 2001; Yager 2012; Pollack 2015). Non-predator signals that an insect may use for risk assessment include alarm calls that could be either directed at the listener or that the listener can eavesdrop upon. For example, insects including termites and honeybees produce vibratory alarm signals to warn their colony members of threats (Hager and Kirchner 2013, Dong *et al.* 2019). These vibrations can either recruit colony members to help defend against the threat or signal to colony members to retreat to safety (Hager and Kirchner 2013, Dong *et al.* 2019). Cues are passive stimuli that have not evolved to change the behaviour of the recipient. The use of acoustic cues by prey to detect enemies and assess risk has been well studied in vertebrates, but less so in insects (reviewed by Yack *et al.* 2020). Predator cues that an insect prey may use for detection or risk assessment include incidental sounds and vibrations that may be produced by moving or foraging. For example, some insects have been shown to respond to low frequency sounds from or similar to flight sounds from parasitoid wasps and flies (Tautz and Markl 1978, White *et al.* 1983, Rothschild and Bergström 1997, Taylor and Yack 2019) and crawling vibrations produced by predators (Guedes *et al.* 2012, Gish 2021).

If predators and parasitoids are producing sounds and vibrations either by communicating with others, or as a by-product of movement, it is reasonable to believe that some insect prey are attending to these sounds and vibrations. While there are some examples of prey insects using vibrations and near-field sounds to detect enemies (e.g., Markl and Tautz 1975, Lohrey *et al.* 2009, Oberst *et al.* 2017, Taylor and Yack 2019), there are few examples for tympanal hearing, aside from detecting the

echolocation calls of bats (Yack *et al.* 2020). Although a number of researchers have proposed that some insects, including crickets, grasshoppers, butterflies, and moths, use tympanal hearing to detect non-bat predators (e.g., Mason 1991; Jacobs *et al.* 2008, Lane *et al.* 2008; Lehmann *et al.* 2010; Fournier *et al.* 2013; Yack *et al.* 2020) there is little experimental evidence for this. Here, I investigate how a species of field cricket (*Gryllus pennsylvanicus*) might use sounds and/or vibrations produced by non-bat predators to assess risk. First, I will review hearing and vibration sensitivity in the genus *Gryllus*, and then I will focus on acoustic communication and sensitivity in my focal species, *Gryllus pennsylvanicus*.

Field crickets of the genus *Gryllus* (Order: Orthoptera, Suborder: Ensifera, Family: Gryllidae) have been studied extensively for their acoustic communication and acoustic sensory systems (e.g., Doherty and Hoy 1985, Hedwig 2006, Hedwig 2016, Shöneich 2020). To date, three types of acoustic receptors are known or proposed: tympanal hearing organs on the foreleg tibia, receptors for solid borne vibrations (i.e., subgenual organs), and near field hearing receptors (i.e., trichoid sensillae on the cerci) (Figure 2.1). Below I review each of these structures, their known or proposed functions, and sensitivities.

Tympanal ears occur on the prothoracic tibia in field crickets and are sensitive to a wide range of frequencies, although most species have peak sensitivity somewhere between 4-6 kHz (Figure 2.1) (Nocke 1972; Paton *et al.* 1977). The function of tympanal hearing in field crickets has been well-studied in the contexts of courtship and mating (reviewed by Hedwig 2006) but the role of tympanal hearing in risk assessment has focused on the detection of ultrasonic bat echolocation. For example, night-flying field

crickets such as *Gryllus bimaculatus* and *Gryllus texensis* exhibit negative phonotaxis in response to ultrasonic echolocation signals produced by bat predators (Nolen and Hoy 1986, Pollack and Martins 2007). In flight-dimorphic species, it has been shown that flight-capable morphs are more sensitive to ultrasound than flight incapable morphs (Pollack and Martins 2007). It has been suggested that field crickets may use non-bat predator sounds to assess predation risk, but to my knowledge there is only one unpublished honours thesis investigating how bird calls affect behaviour in field crickets (Guo 2017).

The subgenual organ in field crickets is located in the tibia on all legs and functions in detection of solid borne vibrations, with sensitivity to frequencies between 0.1 kHz and 1.5 kHz (Figure 2.1) (Eibl 1978, Kühne *et al.* 1984, Lunichkin and Knayzev 2018). While there is no research on the function of the subgenual organ in *Gryllus pennsylvanicus* specifically, vibratory sensitivity has been reported in the context of conspecific communication in a number of Ensiferan species, including *Gryllus* spp.. For example, many species of cricket produce vibrations in the context of aggressive interactions, and there are many studies now suggesting that vibratory signals may play an important role in mating and courtship in crickets (reviewed by Stritih-Peljhan and Virant-Doberlet 2021). It has also been proposed that solid borne vibrations function in risk assessment in field crickets (Dambach 1989, Hedrick 2000, Adamo *et al.* 2013), but to the best of my knowledge this has only been tested once by measuring latency to emerge from a shelter after a series of thumps on the lid of the cricket's experimental arena (see Hedrick 2000).

The cerci in field crickets (*Gryllus* spp.) are located on either side of the posterior end of the abdomen and have trichoid sensillae. While cerci are not typically studied as acoustic receptors in crickets, they have been proposed to detect the near-field components of conspecific communication calls (Dambach 1972, Kämper 1984). One study looking at the involvement of cerci in motor responses to sounds in last-instar nymphs of *Gryllus bimaculatus* suggests that the cerci are sensitive to stimuli between 10 Hz and 1 kHz (Lunichkin and Zhukovskaya 2021). In the context of risk assessment, cerci have largely been studied in the context of wind-elicited escape behaviour in *Gryllus* spp. (Gras and Hörner 1992, Magal *et al.* 2006, Sato *et al.* 2017). In wind-elicited escape, the cerci detect wind created by attacking predators initiating an escape response in the cricket. However, the trichoid sensilla on cerci are also known to detect near-field sound in a number of cricket species (Kämper and Kleindienst 1990). Given that cerci detect near field sound in mating contexts, they may also detect near field sounds such as those produced by flight of aerial insect predators and parasitoids.

Despite crickets having broad acoustic senses, there is a lack of direct evidence showing that they are using their acoustic senses for predator detection and risk assessment. In this chapter of my thesis, I test for acoustically-mediated risk assessment in the fall field cricket, *G. pennsylvanicus*. Below I provide a short overview of the life history traits of *G. pennsylvanicus* that are relevant to predator avoidance followed by the hypotheses and respective predictions that I will be testing in this chapter of my thesis.

Gryllus pennsylvanicus is abundant throughout the northern United States and most of Canada (Weissman and Gray 2019). The species can be found in a variety of

environments, from grassy fields to sandy soils or rocky terrains (Weissman and Gray 2019). Adult fall field crickets are generally present from August through October throughout their whole range, and overwinter in an egg diapause (Murray and Cade 1995; Weissman and Gray 2019). Fall field crickets are primarily crepuscular, meaning that they are most active in the evening and early morning, and retreat into refuges such as crevices or beneath rocks during the day and night (Weissman and Gray 2019). The fall field cricket relies on acoustic communication for mating, with males producing both long and short-range acoustic mating signals. Long-range mating calls are used to broadcast to distant females and signal male quality, whereas short-range courtship calls are typically much quieter and are used when in physical contact with females (Harrison *et al.* 2013). To my knowledge, the only formally documented predators of *G. pennsylvanicus* are wolf spiders (*Tigrosa* spp.) (Storm and Lima 2010). However, it is reasonable to believe that *G. pennsylvanicus* would have similar predators to other *Gryllus* species. Documented predators of other *Gryllus* spp. include scorpions, lizards, bats, rodents, toads, diurnal and nocturnal birds, and bats (Hedrick and Kortet 2006). Table 2.1 lists different cricket species, including *Gryllus* spp., for which predators have been documented, as well as the proposed or known stimuli that evoke antipredator responses. Predators could conceivably produce acoustic stimuli that fall field crickets may use to assess risk, including rustling leaves or grass while foraging, flight sounds, and communication calls. However, despite *G. pennsylvanicus* being prey to an array of species that could produce sounds and vibrations, evidence for acoustically mediated risk assessment in this species is scarce. There is one undergraduate honours thesis investigating how exposure to calls from diurnal bird predators during development

affects growth, development, and behaviour in *G. pennsylvanicus* (Guo 2017). In this thesis, they played back communication calls of diurnally active insectivorous birds to *G. pennsylvanicus* nymphs at dawn and dusk during their development. Once the crickets reached adulthood, they measured latency to exit shelter in a novel environment to investigate if hearing bird calls during development affected adult behaviour. While the trend was non-significant, they found that females exposed to bird predator calls during development exited their shelter in a novel environment faster than females in control treatments (Guo 2017). It is possible, then, that crickets can attend to such sounds and use them to assess risk of predation.

In this thesis chapter, I test the hypothesis that the fall field cricket, *G. pennsylvanicus*, uses sounds and/or vibrations from predators for risk assessment. I tested two predictions: 1) Predators of *G. pennsylvanicus* will produce sounds and vibrations; and 2) Characteristics of these predators' sounds and vibrations will overlap with the sensitivity of *G. pennsylvanicus'* acoustic sensory organs. This hypothesis was tested in an experiment (Experiment 1 of this thesis) in which I characterize sounds and vibrations produced by predators of fall field crickets by analyzing the acoustic characteristics of both pre-recorded predator cues and live recordings of toad predators in seminatural and natural contexts. I then compare these acoustic characteristics of predators with the previously reported sensitivities of *Gryllus* spp. as shown in Figure 2.1.

Table 2.1 Summary of known or proposed predators of crickets (*Gryllus* spp., *Acheta* spp., *Teleogryllus* spp.). Predator species, cricket species, proposed sensory cues used (including chemical, acoustic, touch etc.), and behavioural responses to these cues are included.

Prey species	Stimulus (Stimulus type)	Predator species	Prey response	References
Gryllus pennsylvanicus	Spider predator silk and feces (Chemical stimuli)	Wolf spiders	Freezing Decreased movement	Storm and Lima (2010) Storm and Lima (2008)
Gryllus integer	Vibration from dropping petri dish (Acoustic stimulus)	No suggested simulated predators	Freezing	Hedrick (2013)
Gryllus integer	Exposure to live predator (Visual, chemical, acoustic)	Western toad (*Bufo boreas*)	Freezing Hiding Increased aggression (part of a behavioural syndrome)	Niemelä *et al.* (2012)
Gryllus integer	Exposure to live predator (Visual, chemical, acoustic)	Black widow spider	Freezing	DiRienzo *et al.* (2013)
Gryllus texensis	Air puffs on cerci (Mechanical - wind)	No suggested simulated predators	Escape response (fleeing)	Adamo and McKee (2017)
Gryllus texensis	Ultrasound stimuli (acoustic)	Aerial hawking bats	Negative phonotaxis while in flight	Pollack and Martins (2007)

Table 2.1 continued

Gryllus bimaculatus	Forceps run through leaf litter (acoustic; potentially touch if leaves move) Cricket grasped with forceps (touch)	No suggested simulated predators	Calling ceased Increased latency to emerge in a novel environment Limb autotomized when grasped by forceps	Bateman and Fleming (2006)
Gryllus bimaculatus	Probing of antennae by spider tibia or glass rods (touch)	*Heteropoda venatoria* (spider predator)	Aversion or antennal search (glass rods) Aggression (spider predator tibia)	Okada and Akamine (2012)
Teleogryllus oceanicus	Ultrasound stimuli (acoustic)	Bat species	Negative phonotaxis while in flight	Moiseff *et al.* (1978)
Acheta domesticus	Tapping on refuge with wooden rod (acoustic – sound and vibration) Limb grasped with forceps (touch)	No suggested simulated predator	Limb autotomized when grasped Latency to emerge from refuge following predator event (following tapping and limb grasping)	Wilson *et al.* (2010)
Acheta domesticus	Scent from two insect predators (chemical)	Orange-footed centipede Red-backed spider	Latency to emerge from a refuge increased with centipede markings	Pears *et al.* (2018)
Acheta domesticus	Scent markings of a mammalian predator (chemical)	Shrew species	Latency to begin foraging significantly increased in females, but not males	Tanis *et al.* (2018)

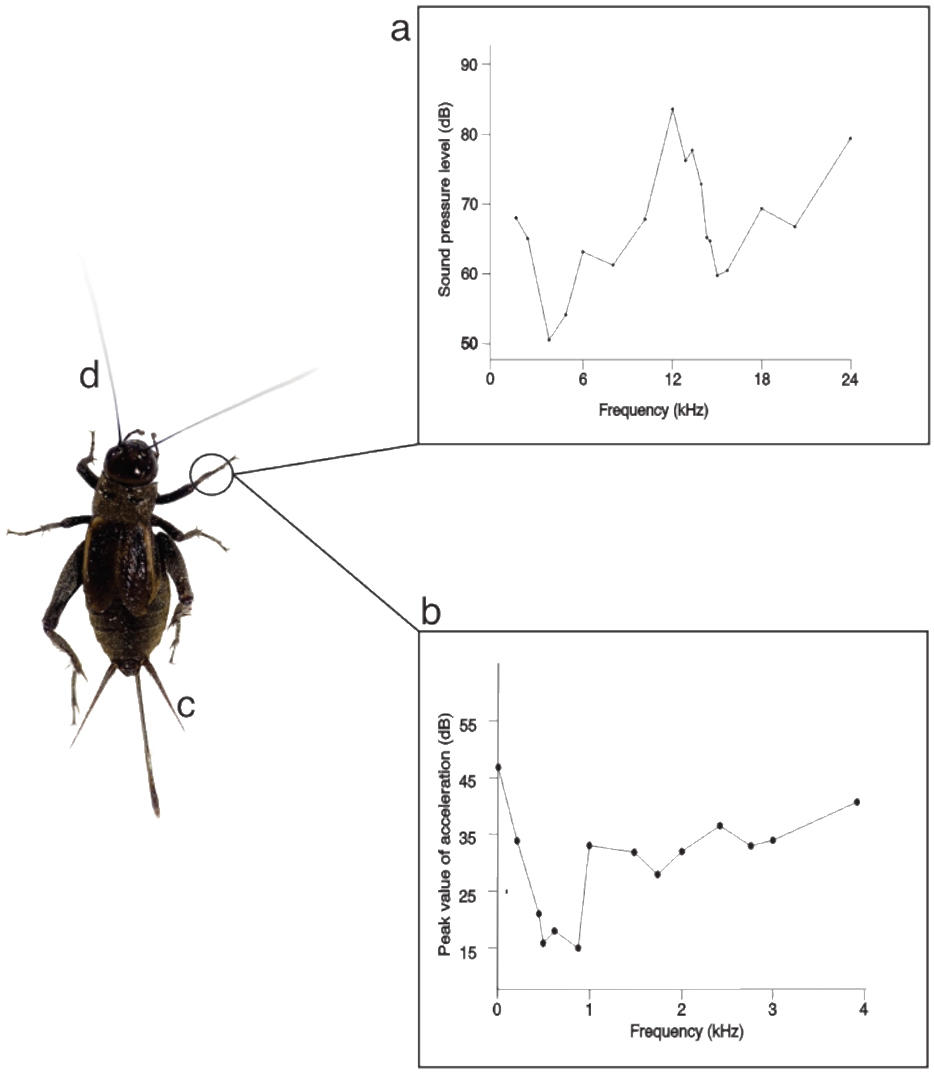

Figure 2.1. Location and sensitivities of *Gryllus* spp. acoustic sensory organs. a) Location of tympanal organ on the foreleg tibia shown on *Gryllus pennsylvanicus,* and whole nerve tuning curve for the tympanal organ in *Gryllus campestris* (tuning curve reproduced from Nocke 1972). b) Location of subgenual organ shown on the foreleg tibia of *Gryllus pennsylvanicus* and whole nerve tuning curve for the foreleg subgenual organ in *Gryllus campestris* (tuning curve reproduced from Nocke 1972). Note that the subgenual organ occurs in the same location on all leg pairs. c) Location of cerci shown on *Gryllus pennsylvanicus* (no tuning curve available). d) Location of antennae shown on *Gryllus pennsylvanicus.* Antennae may be capable of detecting airborne sound however no tuning curve is available.

Methods

Study species

Two live organisms were used in this study – the fall field cricket and the American toad (Figure 2.2). Fall field crickets (*Gryllus pennsylvanicus* Burmeister 1838) were used during recordings of live predator prey interactions with toads. All crickets were obtained from a lab-raised colony at the University of Ottawa, Ottawa, ON, Canada. The colony was originally created in 2010 using wild crickets caught in the University of Koffler Scientific Reserve (Ontario, Canada). Genetic diversity of the colony was maintained by supplementing the laboratory colony annually with ~50 wild caught crickets captured opportunistically in Ottawa (Ontario, Canada). The colony was housed in plastic storage bins (dimensions: 52.1 x 36.8 x 41.9 cm) with adults and juveniles separated. Crickets were provided cardboard egg cartons for shelter, and food (Envigo Teklad Rodent Diet 8604 meal food; www.envigo.com) and water were provided ad libitum. All crickets were housed at 25°C under a 12:12 light/dark cycle, with light hours occurring from 7:00 AM to 7:00 PM. The American toad (*Anaxyrus americanus* Holbrook 1836), a proposed amphibian predator of the fall field cricket, was used to record sounds and vibrations in both lab and natural settings (see recording details below). All toads were captured opportunistically between the hours of 7:00 PM to 10:00 PM on footpaths next to rivers near Carleton University (coordinates: 45.385170, -75.685954) and Andrew Haydon Park (coordinates: 45.350656, -75.816121) in Ottawa, Ontario. Upon capture, toads were placed inside a terrarium (37cm x 22cm x 25cm; Exo-Terra, Montreal, QC, CA) designed for temporary holding of amphibians with a coconut husk substrate (Thrive, Burlington, ON, CA). Prior to

recordings, toads were housed in a larger terrarium (45 cm x 45 cm x 30 cm; Exo-Terra, Montreal, QC, CA) in the lab for a maximum of 24 hours with water provided ad libitum. To ensure that toads would hunt during recordings, they were not provided any food until recordings began. After completion of recordings, toads were released at their capture location. A total of 16 toads including nine males and seven females were captured and used in experiments (see Supplementary Material A). All procedures involving toad capture, housing and experiments were approved (Carleton University Animal Care Committee Protocol no. 117591; Ontario Ministry of Natural Resources collector's permit, Authorization no. 110100).

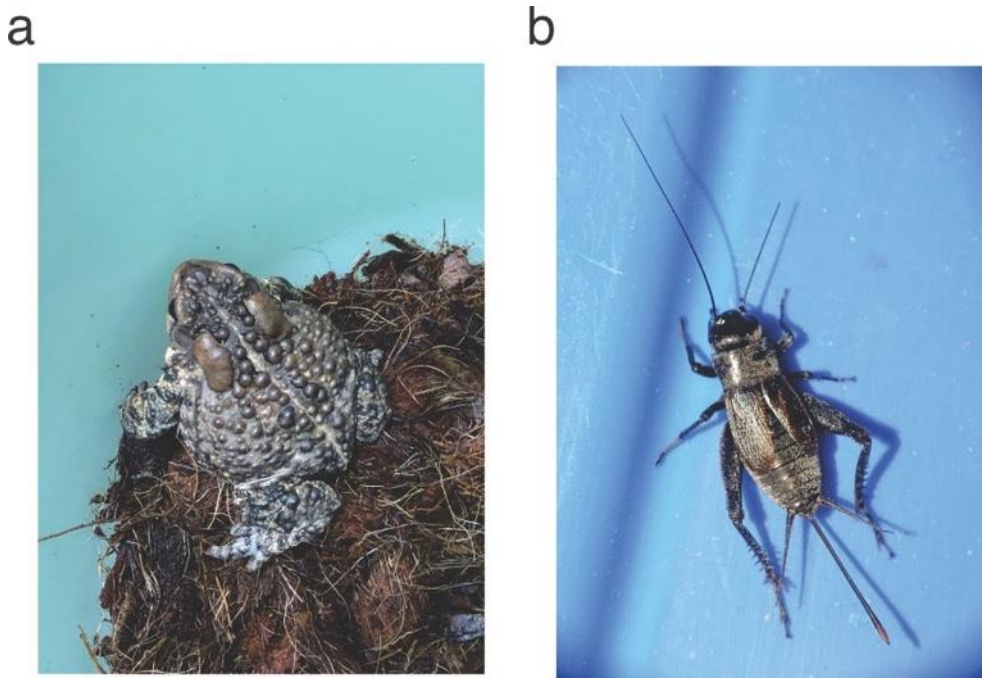

Figure 2.2. Study species used in Chapter 2 and Chapter 3. a) Female American toad (*Anaxyrus americanus*) in lab substrate. Photo taken by E. Brown. b) Female fall field cricket (*Gryllus pennsylvanicus*) in laboratory rearing container. Photo used with permission from M. Osborne.

Recordings in a simulated natural environment (lab)

Sounds, vibrations, and video footage (where applicable) of live or pre-recorded predator sources were recorded in the lab (simulated natural environment) (Figure 2.3). This simulated environment included a glass terrarium (46 x 46 x 30 cm, Exo-Terra, Montreal, QC, CA) filled with layers of volcanic reptile substrate (~3 cm; Exo-Terra Substratum, Montreal, QC, CA), coconut husk (~8 cm; Forest Floor Bedding, Zoo Med, San Luis Obispo, CA, USA) and a mixture of leaf litter, stones, and wood collected outdoors. The terrarium was positioned on an antivibration table in a sound attenuated room (see below) to limit the effects of external vibrations on the recordings. The lid of the terrarium was removed to allow for laser vibrometer recordings (see below). Sounds were recorded using an Earthworks microphone (QTC50, Milford, NH, USA) positioned through an opening on the side of the terrarium. The microphone was connected to a Fostex data recorder (see below for specifications of data acquisition). A sound level meter (Type 2239, B&K, Nærum, Denmark) was placed in the same opening to measure sound levels (dB SPL) directly from the meter during trials. Vibrations were recorded using 2 devices: (1) A laser-Doppler vibrometer (PDV100, Polytec Inc., Irvine, Ca, USA) was used to record vibrations of the substrate by focusing the laser beam on a circular piece of reflective tape (2 mm diameter) fixed to a block of natural wood (approximately 15cm x 10 cm) that was partially buried in the substrate. The analogue output was connected to the Fostex data recorder (see below); (2) a Geophone (GS-20DM 28Hz Oyo Geospace, Houston, TX) was used to record vibrations from the substrate by inserting it into the substrate. The geophone was connected to an amplifier (M-10MX, Edirol/Roland, Los Angeles, CA) and the data recorded to the Fostex

recorder (see below). To record data from the above-mentioned instruments, I used a Fostex data recorder at a sampling frequency of 48 kHz (FR-2 Field Memory Recorder, Boonton, NJ). Data was recorded as .wav files and subsequently transferred to a laptop for further analysis (see analysis section below). Video footage was taken during toad recordings using a Sony Handycam camcorder (hd-rxr520v, Sony, Tokyo, Japan) positioned on a tripod outside of the terrarium. To monitor acoustic events during video recordings and to allow synchronization between sound recordings and behavioural events later, sounds were simultaneously recorded using an external microphone connected to the video camera through the audio port and positioned in the terrarium opening. To synchronize between vibration recordings and behavioural events, the signal from the laser was split between the video camera and the Fostex during live recordings. An audio speaker (Bose Soundlink Color II, Framingham, MA, USA) connected to a laptop was positioned on a tripod outside of the terrarium to play back sounds. The speaker was positioned approximately 20 cm from the microphone on a tripod to ensure that the speaker and microphone were at approximately the same height. Lab experiments were conducted in a sound-attenuated chamber (Eckel Industries Ltd., Cambridge, MA, USA) at Carleton University.

Stimuli of predators used in this experiment were recorded from either live animals or playbacks of pre-recorded stimuli. See Supplementary Material B for a list of these stimuli and their sources. A total of 13 live toads of different sexes were recorded in the lab environment (See details of toads in Supplementary Material A). To record sounds and vibrations generated by live toads, the toad was placed within the terrarium and allowed 30 minutes to acclimate before beginning the recording. All devices were

then turned on to record a baseline prior to introducing 3-5 crickets into the terrarium and were then turned off when all the crickets were captured by the toad, or if the toad showed signs of stress. The toad's mass was taken using a digital balance at the end of the experiment. Supplementary Material A provides details on each captured toad including date and location of capture, sex, mass, and recording location. Sound files of Eastern phoebe (*Sayornis phoebe*) flight, Eastern bluebird (*Sialis sialis*) calls and songs, and breaking branches were also played from a speaker (described above) and recorded using the microphone, geophone, and laser (described above) (see Supplementary Material B).

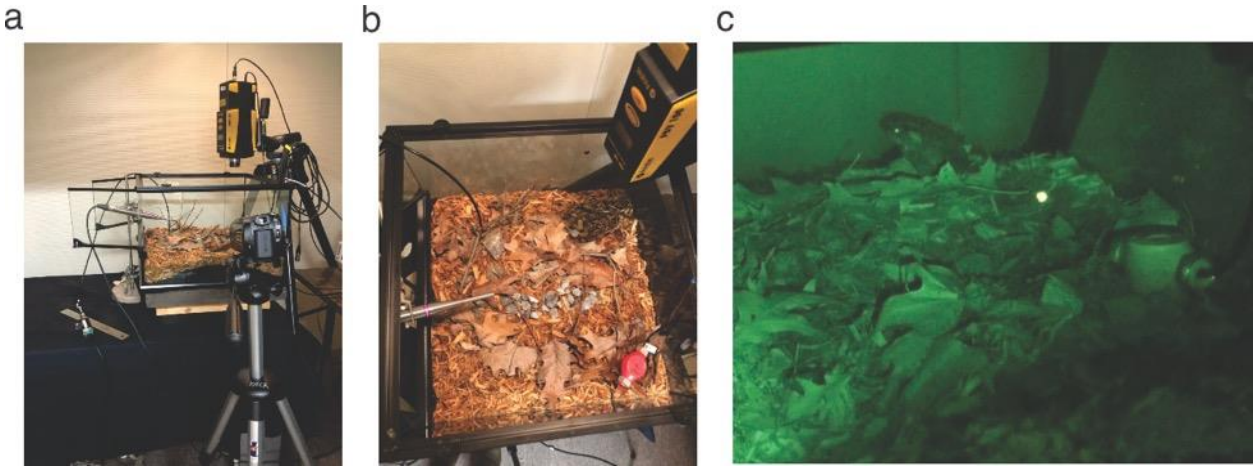

Figure 2.3. Lab set up for live recordings and playbacks of predator signals and cues used in Chapter 2. a) Overview of equipment set-up showing the recording terrarium, earthworks microphone, laser vibrometer and video camera. b) Close up of recording terrarium showing the microphone inserted through and opening in the terrarium, the geophone (red device in bottom right) embedded in the substrate, and the laser vibrometer. The laser beam is focused on reflective tape on a piece of mulch within the substrate. c) Screenshot from video footage of a live recording of toad vibrations using the laser vibrometer and geophone. The laser beam is focused on reflective tape on a piece of mulch within the substrate.

Recordings in a natural (field) environment

In addition to the lab recordings described above, sounds and vibrations from a subset of three different toads were recorded in a natural environment (see Supplementary Materials A and B for more details). These recordings were performed from September 20th to October 15th, 2022 in two different regions where field crickets occurred, and on two different substrates: 1) On a grassy substrate with little anthropogenic noise near Maberly, ON; and 2) On leaf litter in a natural space near the downtown core in central Ottawa, ON (Figure 2.4). On the first substrate, two toads (one male and one female) were recorded, and one female was recorded on the second substrate. For these toad recordings, I used a custom-made plexiglass box (45 cm length x 45 cm width x 45 cm height) with an opening to allow easy placement of recording equipment in the recording arena (Figure 2.4). Sounds and vibrations were recorded using the same sensors (i.e. microphone, laser and geophone) as described for lab recordings above, with slight modifications to the procedure (see details below). The laser and microphone were connected simultaneously to the XLR inputs of a Canon XA15 video camera to record data, and the geophone was connected to a Fostex data recorder. Video footage was simultaneously recorded to determine which behaviours produced the recorded sounds and vibrations. A canopy was placed over the recording area to protect equipment from potential rain (see Figure 2.4). Once the recording arena was set up, a toad was placed inside the arena and allowed to acclimate for a minimum of 5 minutes. After this acclimation period, the recording equipment was turned on and a few locally captured insects, including *G. pennsylvanicus*, were placed inside the recording arena to stimulate hopping and prey capture in the toad.

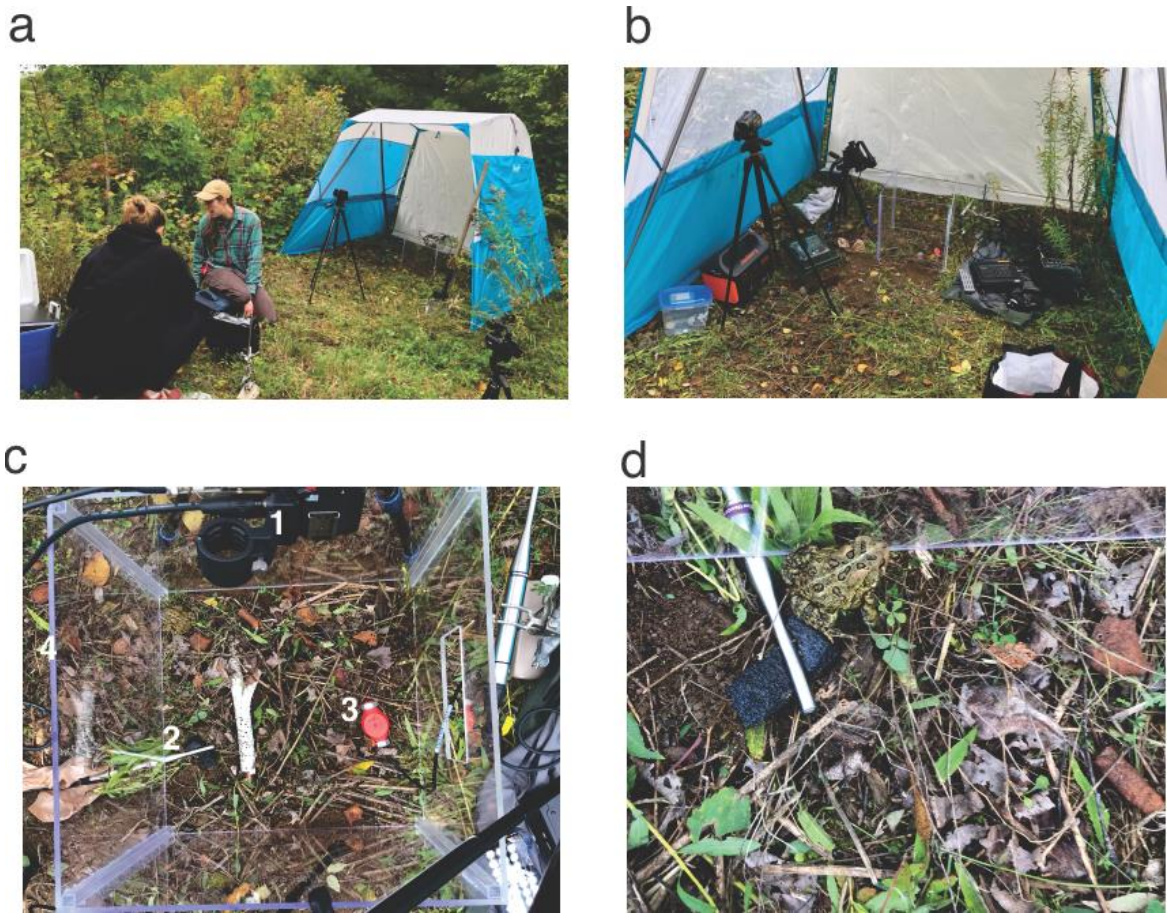

Figure 2.4. Field set-up for live recordings of American toads (*Anaxyrus americanus*) in a cricket's natural habitat. a) Overview of a location used for field recordings. Shown here is the tent used to protect equipment from the elements. b) Overview of recording set-up inside the tent. c) Overview of equipment used for field recordings. 1. The camera used to record video footage and data from the laser and microphone during field recordings. 2. Microphone used to record airborne sound from toads. 3. Geophone positioned within the substrate to record vibrations. 4. Experimental arena used for field recordings. Not shown is the laser vibrometer positioned above the arena with the laser beam focused on reflective tape on the birch branch. d) Close-up of substrate used for field recordings with a mix of grass, leaf litter, twigs, and stones.

Analysis of acoustic stimuli

Acoustic stimuli were analyzed in the spectral, amplitude, and time domains. Figure 2.5 provides an illustration of which measurements were taken. Spectral features were measured from a power spectrum, and included first peak frequency, second peak frequency, and bandwidth below the first and second peaks. All spectral measurements were computed using Raven Pro 1.6 (Cornell Laboratory of Ornithology, Ithaca, New York). Power spectra and spectrograms were generated using a 4096-point fast Fourier transform (Hann window, 50% overlap). First and second peaks were defined as the peaks with the most and second most the power spectrum respectively. Bandwidths were measured at 10 dB below the first and second peaks. To account for the shoulder (i.e., a peak of energy that occurs in the lower frequency range that reflects low frequency noise of the recording equipment or background noise in the building) found in some recordings, I measured the bandwidth on the left and right side of the peaks separately to allow a bandwidth measurement to still be measured (see Figure 2.5). Amplitude measurements included peak amplitude and root mean square (RMS) amplitude. Amplitude measurements were computed using R Studio (R Studio Team 2020). Peak amplitude was defined as the maximum deviation of the waveform from zero (i.e., the higher absolute value of the maximum or minimum amplitude). Root-mean-square amplitude is calculated by taking the square root of the average of all measured squared amplitudes. All amplitude measurements were measured for both the stimulus and a background sample of the same duration as the stimulus, and the difference between stimulus and background amplitude was taken. This allows for stimulus amplitude to be compared to background to see if the stimulus amplitude is

higher than background to establish a basis for comparison of amplitude values between different stimuli. Time domain measurements included duration and duty cycle. Stimulus duration, defined as the amount of time in seconds elapsed during a single instance of the stimulus, was measured using R Studio. Duty cycle was measured manually in Raven as the percentage of time where there was sound energy present from the stimulus in a period of 1.2 seconds (i.e., the longest duration of all measured sound stimuli). Sounds or vibration samples used for acoustic measurements were chosen after visual inspection using Raven. For each sound and vibration, three representative samples were chosen from the original .wav files by assessing the file to find the sound or vibration with the best signal-to-noise ratio.

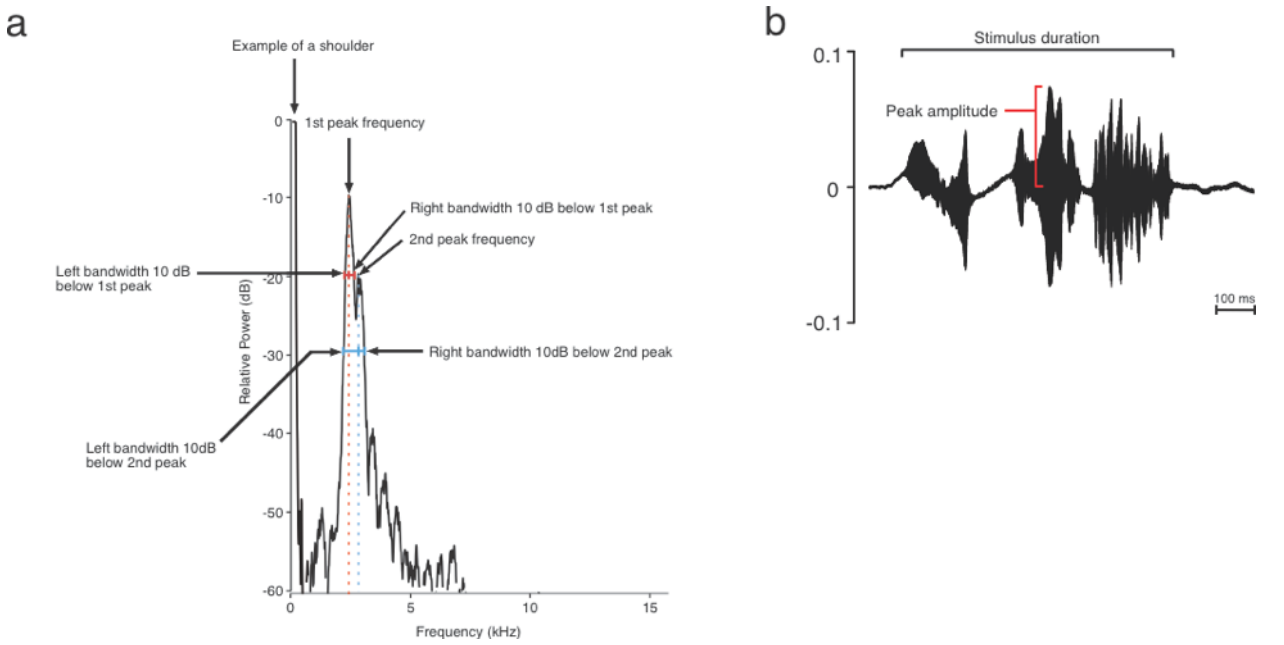

Figure 2.5. Acoustic traits measured in this study, using a sound file of an Eastern bluebird song. This also provides an example of the “shoulder” produced in many sound recordings. Shoulders were not considered a peak frequency if they were present in both background and sound stimulus samples. a) Example of all measured spectral traits using a sound file of an Eastern bluebird song. b) Example of measured amplitude characteristics using the same sample of an Eastern bluebird song. Note that root mean square amplitude is not shown here.

Results

Characterization of acoustic stimuli

In this chapter of my thesis I asked, what sounds and vibrations (produced either by live predators or through playbacks of pre-recorded predator sounds) are potentially available to a cricket, as assessed in both a lab and field enclosure. I also asked, how might these stimuli overlap with cricket hearing and vibratory sensitivity, as assessed by comparing the spectra of these stimuli with sensitivity curves available in the literature. In Chapter 3 of my thesis (see below), I play back these acoustic signals and cues to crickets to assess how they respond.

What sounds are vibrations are potentially available to a cricket (simulated natural environment)?

All predator stimuli recorded in a semi-natural, lab environment generated both airborne sounds and solid borne vibrations (see Table 2.2, Figures 2.6-2.10). The acoustic characteristics between stimulus types varied in several respects. Also, the acoustic features within stimulus types varied depending on the type of recording device, among other factors. First, I review each stimulus source and describe the sounds and vibrations that were recorded using different instruments. Then I comment on differences between different stimuli, and how the features compare to a calibrated stimulus, the mass drop.

Recordings from live toads

While recording live toads, sounds and vibrations resulted from four types of behaviours: 1) crawling when exploring the arena; 2) hopping in pursuit of a prey or exploring the arena; 3) a vocalization (“release call”) while being handled; and 4) a vibration (“release vibrations") while being handled.

Toad hopping

While toads did crawl while initially exploring the arena, they largely hopped in pursuit of cricket prey. As such, this section discusses the acoustic characteristics of sounds and vibrations produced by toad hopping. Recorded toad hops described in this section occurred on cypress mulch substrate with a thin layer of leaf litter on the surface. Live recorded toad hops on average produced low amplitude sounds (stimulus peak amplitude: (peak amplitude above background: 295.57 U ± 334.11) (means ± sd) (Table 2.2, Figures 2.6, 2.7, 2.9). Peak frequencies of toad hop sounds were quite low (893.55 Hz ±1287.97, n=7) and broadband (bandwidth measured left of peak: 117.62 Hz ± 56.07; bandwidth right of peak: 98.97 Hz ± 49.65, n=7) (Table 2.2, Figures 2.6, 2.7, 2.9). Toad hopping sounds were highly variable in peak frequency, with some toads producing peaks around 200 Hz and others producing peaks closer to 3 kHz. Sounds from toad hops also had energy extending across a wide range of frequencies, with significant energy from < 1 kHz – 15 kHz (Figure 2.10, Table 2.2). Laser recordings of toad hopping were relatively high amplitude compared to background (peak amplitude: 1680.14 U ± 990.2) Vibrations produced by toad hopping were all very low frequency (peak frequency of laser recordings: 17.57 Hz ± 11.72, n=7) and broadband (bandwidth

right of peak in laser recordings: 30.16 Hz ± 30.92, n=7) (Table 2.2, Figures 2.6, 2.7, 2.9).

In addition to measuring the acoustic characteristics of toad hopping sounds and vibrations, I assessed if amplitude of hops varied depending on toad mass using a linear regression. The model showed that amplitude of sounds and vibrations did not vary significantly between toads of different masses (p=0.369, n=7, Figure 2.6).

Toad vocalizations and vibrations

When being handled, toads produced a vocal signal likely to be a release call (see discussion). Both sexes produced release calls only while being handled, especially when light pressure was applied to the sides of their abdomen. These calls were high amplitude compared to background (peak amplitude: 856 U ± 238.39) with first peak frequency of recorded calls generally ranging from 1500-1700 Hz with energy highly concentrated around the peak (Figure 2.7, Table 2.10).

Toads also produced a vibrational signal while being handled, likely to be release vibrations. While release vibrations and calls seemed to be separate signals, they were always produced simultaneously in my experiment. Release vibrations recorded by the laser were high in amplitude compared to background (peak amplitude: 352.67 U ± 15.31) with first peak frequencies between 50-100 Hz. Release vibrations recorded by the geophone were lower in amplitude, but still distinguishable above background (peak amplitude: 154.0 U ± 48.14).

Recordings of breaking branches

Microphone recordings of playbacks of breaking branches over a loudspeaker were high in amplitude compared to background (peak amplitude: 2170.33 U ± 126.75; Figure 2.9a, 2.10; Table 2.2). These sounds had higher first peak frequencies (5976.80 Hz ± 862.48, n=3) and narrower bandwidths compared to the other recorded predator sounds (bandwidth measured left of peak: 74.91 Hz ± 25.11, bandwidth measured right of peak: 58.42 Hz ± 35.22, n=3) (Figure 2.9a; 2.10; Table 2.2). Playbacks of breaking branch sounds generated some low amplitude substrate vibrations detectable by the laser vibrometer. These vibrations were very low amplitude, but still slightly higher than background amplitude recordings (peak amplitude: 147.33 ± 85.17, n=3). Vibrations recorded by the laser were very low frequency and relatively broadband (peak frequency: 27.39 Hz ± 13.66, bandwidth measured right of peak: 28.54 Hz ± 5.91, n=3) (Figure 2.9a; 2.10; Table 2.2). Vibrations transferred to the substrate from playbacks of breaking branch sounds were not detectable by the geophone.

Recordings of Eastern phoebe flight

Playbacks of Eastern phoebe flight sounds recorded by the microphone were high in amplitude compared to background (peak amplitude: 1063.33 ± 121.33 U). Flight sounds recorded by the microphone were very low frequency, with first peak frequencies at 70.53 ± 0.185 Hz (n=3) and relatively broadband (bandwidth left of peak: 13.88 ± 0.22 Hz, bandwidth right of peak: 44.30 ± 2.19 Hz) (Figure 2.9b; 2.10; Table 2.2). Despite microphone recordings of flight sounds having very low first peak frequencies, there was significant energy extending up to around 7 kHz in all

microphone recordings of Eastern phoebe flight (Figure 2.9b; 2.10). Playbacks of Eastern phoebe flight sounds also transferred some vibrations to the substrate that were detectable by the laser vibrometer (peak amplitude: 209.33 U ± 39.55). Laser recordings of phoebe flight playbacks had first peak frequencies of 117.18 Hz ± 20.29 Hz and were quite broadband (bandwidth left of first peak: 37.58 Hz ± 23.7; bandwidth right of first peak: 51.55 Hz ± 19.46) (Figure 2.9b; 2.10; Table 2.2). Vibrations transferred to the substrate from playbacks of Eastern phoebe flight were not detectable by the geophone (Figure 2.9b).

Recordings of Eastern bluebird song

Playbacks of Eastern bluebird songs recorded by the microphone were high in amplitude compared to background (peak amplitude: 1930.67 U ± 208.87, n=3) (Table 2.2). Bluebird song playbacks had first peak frequencies of around 2 kHz with very little variation between different recordings (first peak frequency: 2238.27 Hz ± 30.99, n=3) and had energy highly concentrated at the peak (bandwidth left of peak: 170.81 Hz ± 45.87, bandwidth right of peak: 148.31 Hz ± 68.29, n=3) (Figure 2.9b; 2.10; Table 2.2). Playbacks of Eastern bluebird songs transferred some low amplitude vibrations to the substrate that were detectable by the laser vibrometer, however the laser detected both vibrations and airborne sound in recordings (peak amplitude: 6 U± 4.36). The peak frequency of laser recordings of bluebird calls was highly variable, depending on how strongly the laser detected airborne sound produced by the playback, (first peak frequency: 1105.56 Hz ± 1754.21). First peak frequencies of laser recordings of bluebird song playbacks were also quite narrowband (bandwidth left of peak: 26.17 Hz ± 15.61,

peak frequency right of peak: 22.06 Hz ± 8.98, n=3). Substrate vibrations generated by playbacks of Eastern bluebird songs were not detectable by the geophone.

Recordings of Eastern bluebird call

Playbacks of Eastern bluebird calls were also high in amplitude compared to background noise (peak amplitude: 1358.67 U ± 852.05, n=3). Amplitudes varied substantially depending on the recording played back. Bluebird calls had an average first peak frequency of about 3700 Hz with energy highly concentrated around the peak (bandwidth left of peak: 60.76 Hz ± 53.131, bandwidth right of peak: 68.72 Hz ± 61.38) (Figure 2.9b; 2.10; Table 2.2). Playbacks of bluebird calls transferred some vibrations to the substrate that were detectable by the laser. Transferred vibrations were quite low in amplitude compared to background (peak amplitude: 49.67 U ± 67). The peak frequency of laser recordings of bluebird calls was highly variable, depending on how strongly the laser detected airborne sound produced by the playback (1105.56Hz ± 1754.21) and narrowband (bandwidth left of peak: 26.17 Hz ± 15.61, peak frequency right of peak: 22.06 Hz ± 8.98, n=3) (Figure 2.9b; 2.10; Table 2.2). Substrate vibrations transferred from playbacks of bluebird songs were not detectable by the geophone.

Recordings from Mass Drop

To document the natural resonance of the substrate used for recordings, I also recorded sounds and vibrations produced by dropping a known mass from a height of 10 cm. Microphone recordings of mass drops were very high amplitude compared to background (peak amplitude: 7845192 U ± 21613.92). Generally, microphone

recordings of mass drops had high first peak frequencies (13734.38 Hz ± 3457.01) with substantial energy extending above 20 kHz. Laser recordings of mass drops were also high amplitude compared to background (peak amplitude: 721741 U ± 68921.48). Vibrations from mass dropping recorded by the laser were very low frequency (first peak frequency: 109.38 Hz ±13.53). Geophone recordings of mass drops were lower amplitude compared to laser recordings, but still quite high compared to background (peak amplitude: 87508.33 U ± 8937.15). Geophone recordings of vibrations from mass drops were similar in peak frequency to laser recordings, with first peaks below 100 Hz.

Summary of sound and vibration recordings

All proposed predators of *Gryllus pennsylvanicus* produced sounds and/or vibrations that are potentially accessible to insects for risk assessment. Predator sounds did vary substantially between sources in both amplitude and spectral characteristics. Generally, played back sounds were higher in amplitude compared to background than live recordings of toads, although all predator sounds were above background noise. Predator sounds also varied substantially in frequency, with toad hopping and played back bird flight sounds having much lower first peak frequencies (<1000 Hz) than the other recorded stimuli. These amplitude and spectral differences suggest that predator sounds are potentially distinguishable by insect hearing.

Vibrations produced by predators were also available to insect prey for risk assessment. The strongest vibrations were produced by live recordings of toad hopping. Other vibrations measured were transferred to the substrate by playbacks of pre-recorded bird flight and bird communication signals, but these vibrations were generally

very low amplitude. Vibrations generally did not differ substantially in spectral characteristics except for when the laser vibrometer detected airborne sound during playbacks. As vibrations differ in terms of amplitude, it is possible that they are also distinguishable by insect hearing.

Comparison of acoustic stimuli to cricket acoustic sensory organ sensitivity

I assessed how the stimuli discussed above overlapped with the sensitivity of field cricket acoustic sensory organs. To achieve this goal, I asked two questions: 1) Of those recorded sound stimuli with a high signal to noise ratio (i.e. were detectable above noise), how did these overlap with the sensitivity of field cricket tympanal organs? and 2) Of the recorded vibration stimuli with a high signal to noise ratio, how did these overlap with the sensitivity of the field cricket subgenual organ? Extent of overlap between the power spectrum of the stimulus with the whole nerve tuning curve of the cricket was assessed qualitatively by overlapping the two curves to assess whether the dominant frequency of the stimulus overlapped with the most sensitive region of the tuning curve.

Table 2.2 continued

Table 2.2. Summary of acoustic measurements taken for all recorded predator stimuli. Means ± standard deviation are shown.

Stimulus	Recording Device	1st Peak Frequency (Hz)	Bandwidth 1st Peak (Left) (Hz)	Bandwidth 1st Peak (Right) (Hz)	2nd Peak Frequency (Hz)	Bandwidth 2nd Peak (Left) (Hz)	Bandwidth 2nd Peak (Right) (Hz)	Peak Amplitude Over Background (U)	Root Mean Square Amplitude Over Background (U)	Duration (s)	Duty Cycle (%)
Toad Hop	Earthworks microphone	766.69 ± 1287.97	125.52 ± 56.07	80.87 ± 49.65	1846.64 ± 1400.93	194.62 ± 52.11	85.46 ± 49.45	295.57 ± 334.11	-5.36 ± 75.89	0.38 ± 0.06	31.25 ± 4.64
Toad Hop	Laser vibrometer	40.15 ± 11.72	NA	30.16 ± 30.92	70.32 ± 20.28	37.52 ± 4.84	47.89 ± 9.09	1680.14 ± 990.2	322.14 ± 162.28	0.37 ± 0.09	31.04 ± 7.34
Toad Hop	Geophone	26.91 ± 25.32	59.18 ± 17.17	81.1 ± 13.35	70.33 ± 23.45	89.45 ± 38.29	65.09 ± 10.87	505.14 ± 442.15	72.09 ± 59.79	0.37 ± 358.26	31.04 ± 3.58
Branch Break	Earthworks microphone	5976.8 ± 862.48	74.91 ± 25.11	58.42 ± 35.22	4515.62 ± 2233.84	137.24 ± 27.42	110.44 ± 68.19	2170.33 ± 126.75	94.22 ± 50.06	0.14 ± 0.01	11.39 ± 0.96
Branch Break	Laser vibrometer	27.39 ± 13.66	31.82 ± 4.26	28.54 ± 5.91	179.66 ± 130.55	30.69 ± 0.3	47.32 ± 37.78	147.33 ± 85.17	19.6 ± 31.13	0.13 ± 0.02	10.83 ± 1.44
Branch Break	Geophone	43.45 ± 18.55	NA	29.21 ± 17.64	NA	NA	NA	1 ± 2.65	-0.03 ± 0.35	0 ± 0	0 ± 0
Eastern Phoebe Flight	Earthworks microphone	70.53 ± 0.19	13.88 ± 0.22	44.3 ± 2.19	136.7 ± 27.02	86.61 ± 28.37	44.66 ± 23.67	1063.33 ± 121.33	103.74 ± 11.13	1.05 ± 0	87.5 ± 0
Eastern Phoebe Flight	Laser vibrometer	117.18 ± 20.29	37.58 ± 23.7	51.55 ± 19.46	136.74 ± 165.85	35.4 ± 10.11	98.94 ± 63.47	209.33 ± 39.55	36.44 ± 5.68	0.97 ± 0.03	80.56 ± 2.41
Eastern Phoebe Flight	Geophone	93.79 ± 0.09	NA	106.71 ± 6.96	NA	NA	NA	41.33 ± 35.64	5.74 ± 0.17	0.96 ± 0.05	80 ± 4.41
Bluebird Song	Earthworks microphone	2238.27 ± 30.99	170.81 ± 45.87	148.31 ± 69.29	2273.43 ± 263.85	232.03 ± 284.84	315.42 ± 52.48	1930.67 ± 208.87	375.64 ± 63.2	0.65 ± 0.09	54.17 ± 7.22

Table 2.2 continued

Bluebird Song	Laser vibrometer	746.45 ± 1272.41	60.16 ± NA	95.62 ± 108.67	2250.63 ± 47.21	93.9 ± 51.73	211.21 ± 87.31	21.67 ± 77.51	-16.23 ± 18.47	0.74 ± 0.02	61.55 ± 1.87
Bluebird Song	Geophone	58.94 ± 0.32	16.71 ± 5.44	16.61 ± 3.29	15.81 ± 6.64	NA ± NA	47.91 ± 27.06	233.33 ± 297.65	-0.13 ± 0.42	0 ± 0	0 ± 0
Bluebird Call	Earthworks microphone	3703.1 ± 213.21	60.76 ± 53.12	68.72 ± 61.38	3949.23 ± 246.94	147.96 ± 123.72	80.77 ± 45.09	1358.67 ± 852.05	136.33 ± 110.99	0.07 ± 0.01	5.56 ± 0.96
Bluebird Call	Laser vibrometer	1105.56 ± 1754.21	26.17 ± 15.61	22.06 ± 8.99	871.25 ± 1255.63	45.83 ± 48.74	45.1 ± 53.29	49.67 ± 67	13.16 ± 20.45	0.04 ± 0.01	3.69 ± 0.63
Bluebird Call	Geophone	58.77 ± 0.45	13.68 ± 0.6	13.99 ± 0.21	70.47 ± 91.6	13.71 ± 6.8	32.81 ± 21.95	6 ± 4.36	0.19 ± 0.19	0 ± 0	0 ± 0
Mass Drop	Earthworks microphone	13734.38 ± 3457.01	113.02 ± 22.63	99.13 ± 24.82	13226.63 ± 3192.78	137.48 ± 31.88	238.42 ± 187.39	7845192 ± 21613.92	357347.68 ± 249571.36	0.14 ± 0.02	11.53 ± 1.58
Mass Drop	Laser vibrometer	109.38 ± 13.53	100.89 ± 10.87	118.17 ± 40	9070.15 ± 129.27	167.2 ± NA	218.86 ± NA	721741 ± 68921.48	140266.2 ± 35754.36	0.18 ± 0.04	14.72 ± 3.37
Mass Drop	Geophone	70.31 ± 0	75.34 ± 1.68	101.92 ± 1.73	3687.65 ± NA	166.14 ± NA	247.76 ± NA	87508.33 ± 8937.15	21375.9 ± 2040.93	0.18 ± 0.05	15 ± 3.85

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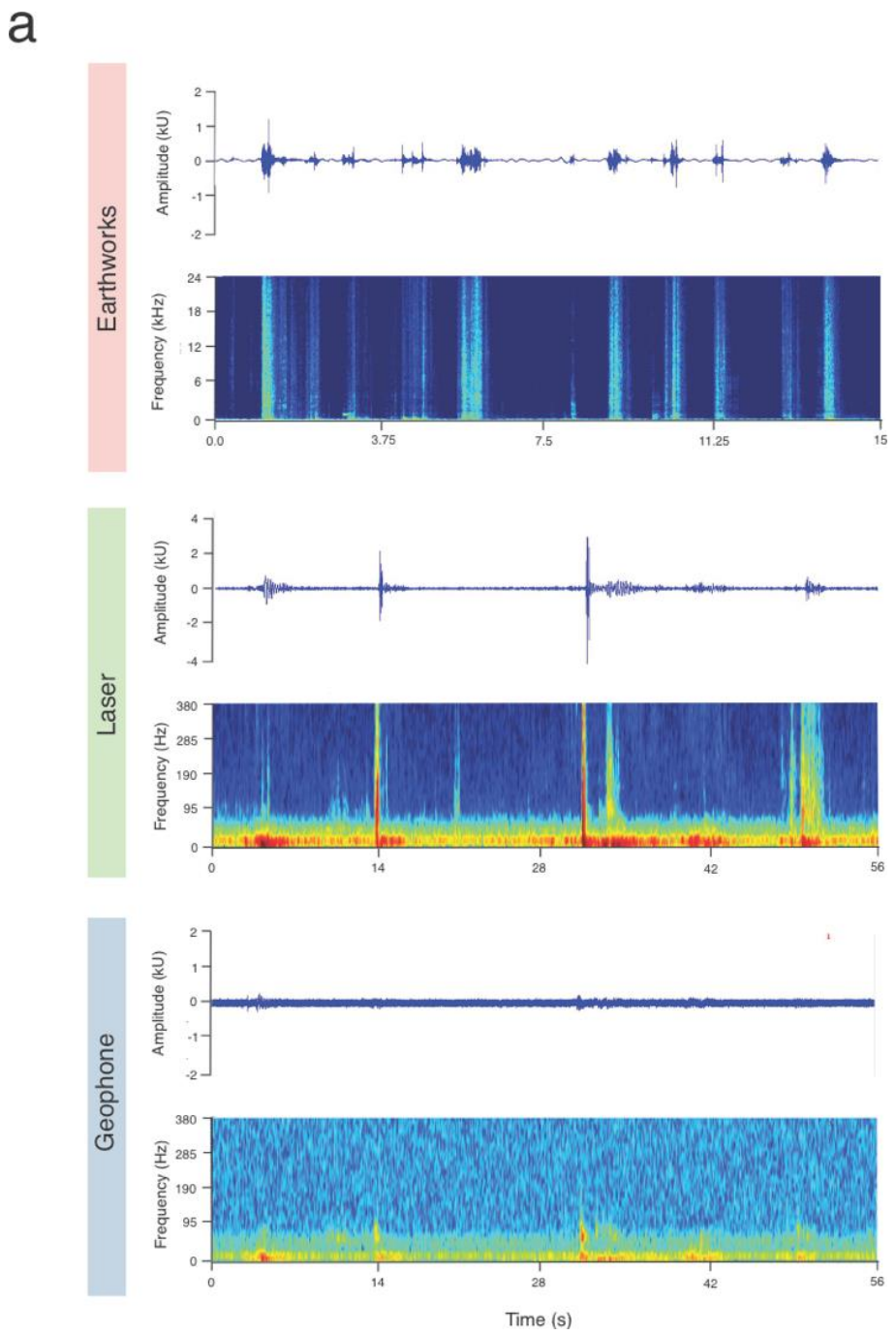

Figure 2.6. a) Waveforms and spectrograms for an extended toad hopping sequence recorded in the lab. Laser and geophone recordings were extracted from the same recording session, but the Earthworks recording was taken from a separate recording session. All spectrograms were generated using a 4096 FFT (50% overlap, Hann window).

Figure 2.6 continued

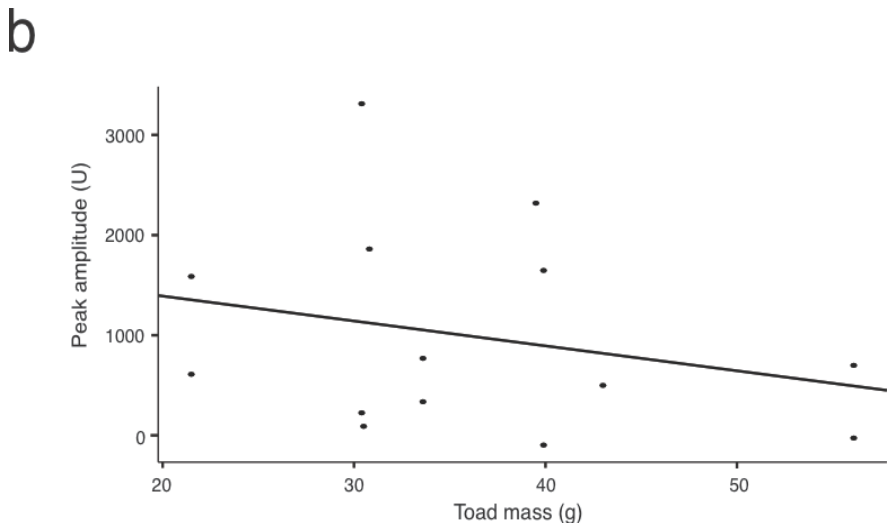

Figure 2.6. b) Regression showing the relationship between toad mass (g) and peak amplitude of the recording sample.

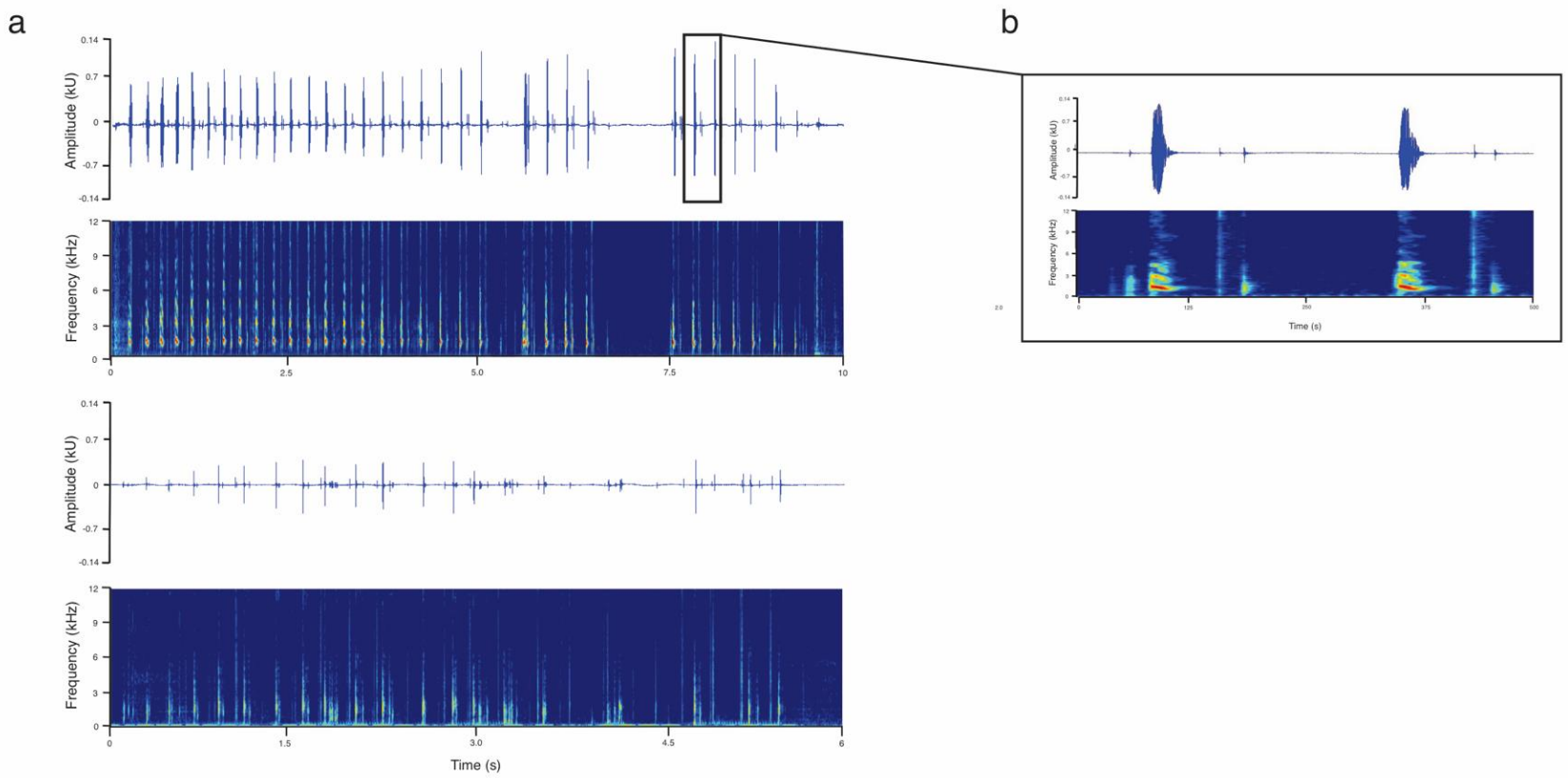

Figure 2.7. Waveforms and spectrograms of airborne sound from release calls generated by the American toad (*Anaxyrus americanus*). a) Waveforms and spectrograms of longer samples of release calls from two individuals. b) Waveform and spectrogram of a small subsample of the release call produced by the individual in the top two panels of section a.

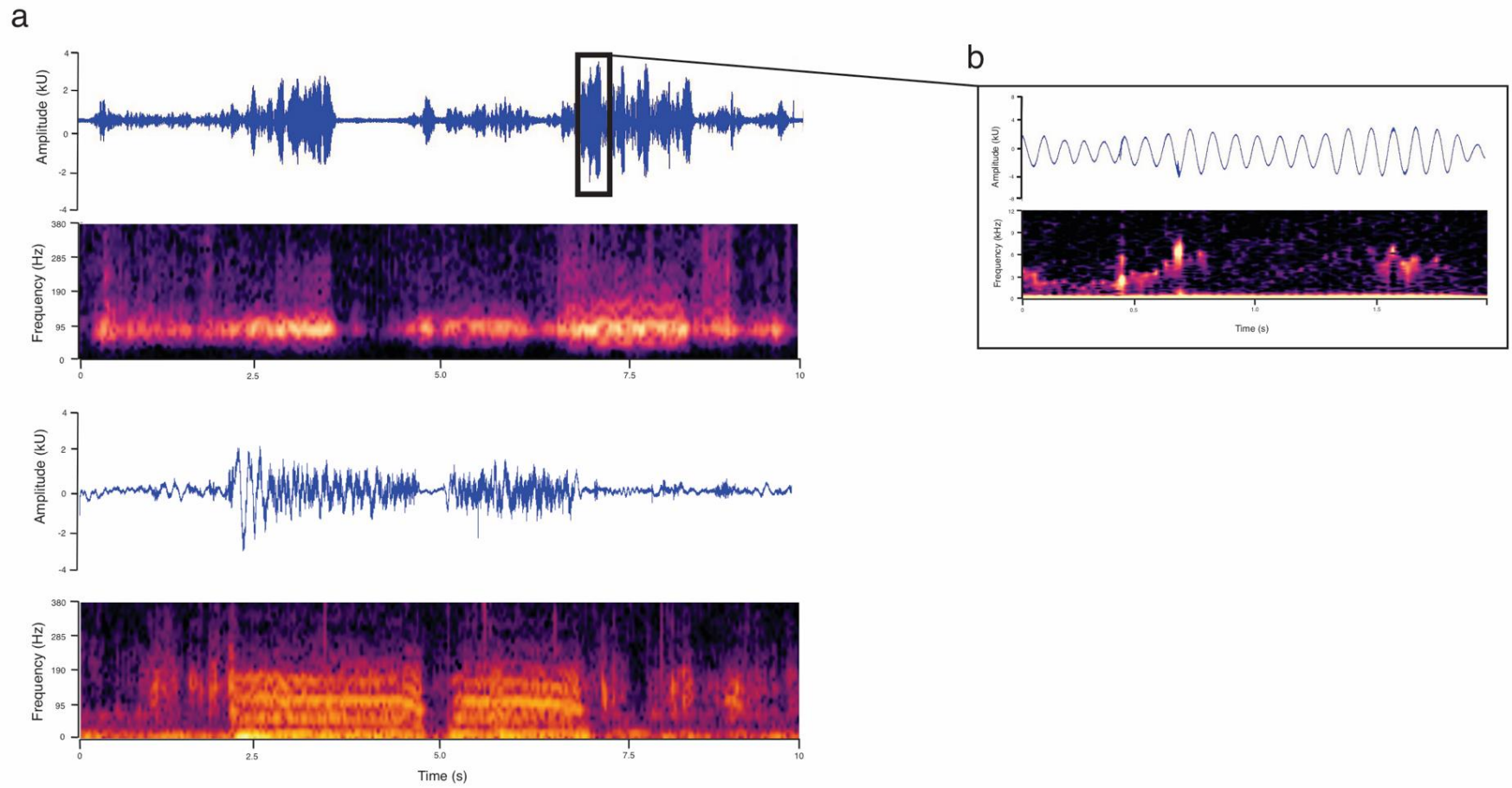

Figure 2.8. Waveforms and spectrograms for laser recordings of release vibrations generated by American toads (*Anaxyrus americanus*). a) Waveforms and spectrograms from a longer sample of release vibrations recorded from two different individuals. b) Waveform and spectrogram from a shorter subsample of the recording in the top two panels of section a.

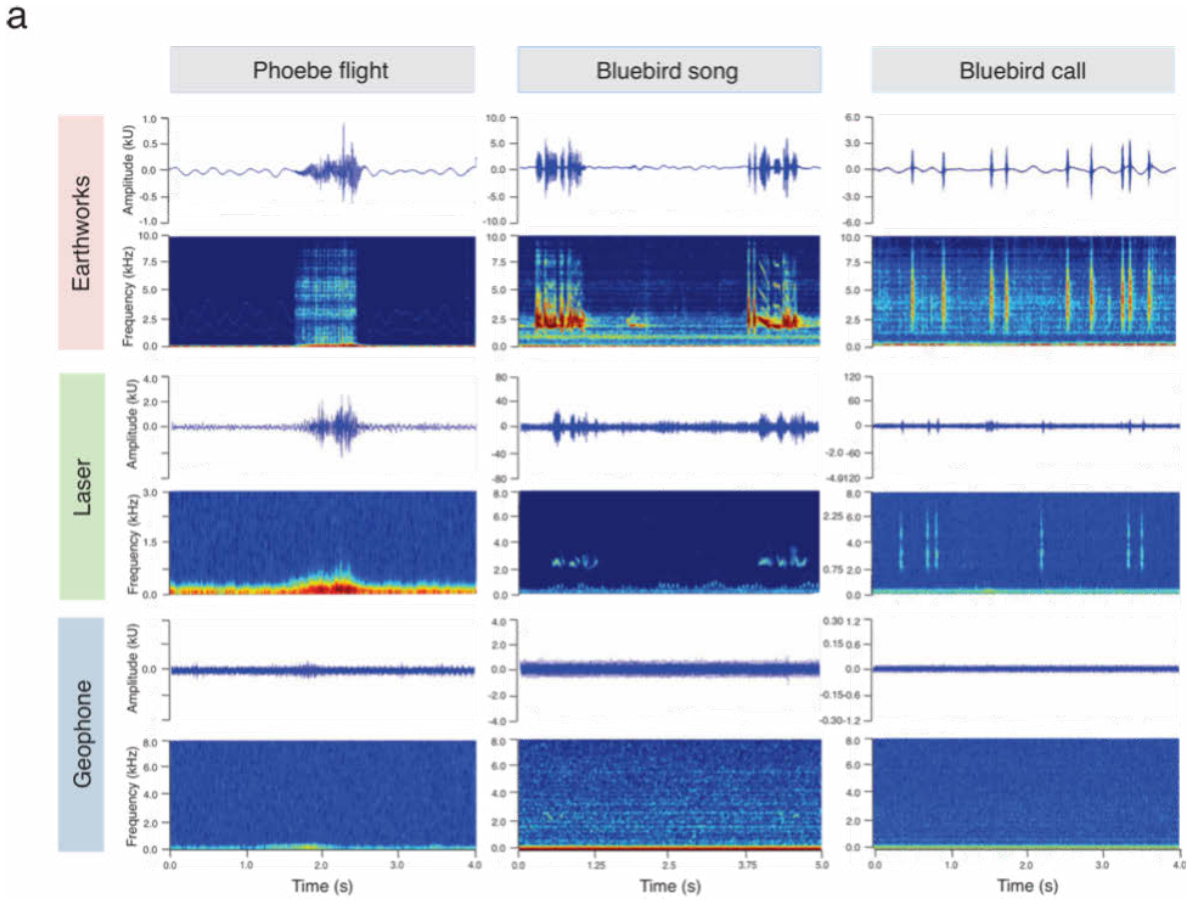

Figure 2.9. Extended waveforms and spectrograms for representative recordings of predator sounds and vibrations. Recordings were taken using an Earthworks microphone, laser vibrometer, and geophone. All spectrograms were generated using a 4096 FFT (50% overlap, Hann window). a) Sounds and vibrations from playbacks of recordings of avian predators.

Figure 2.9 continued

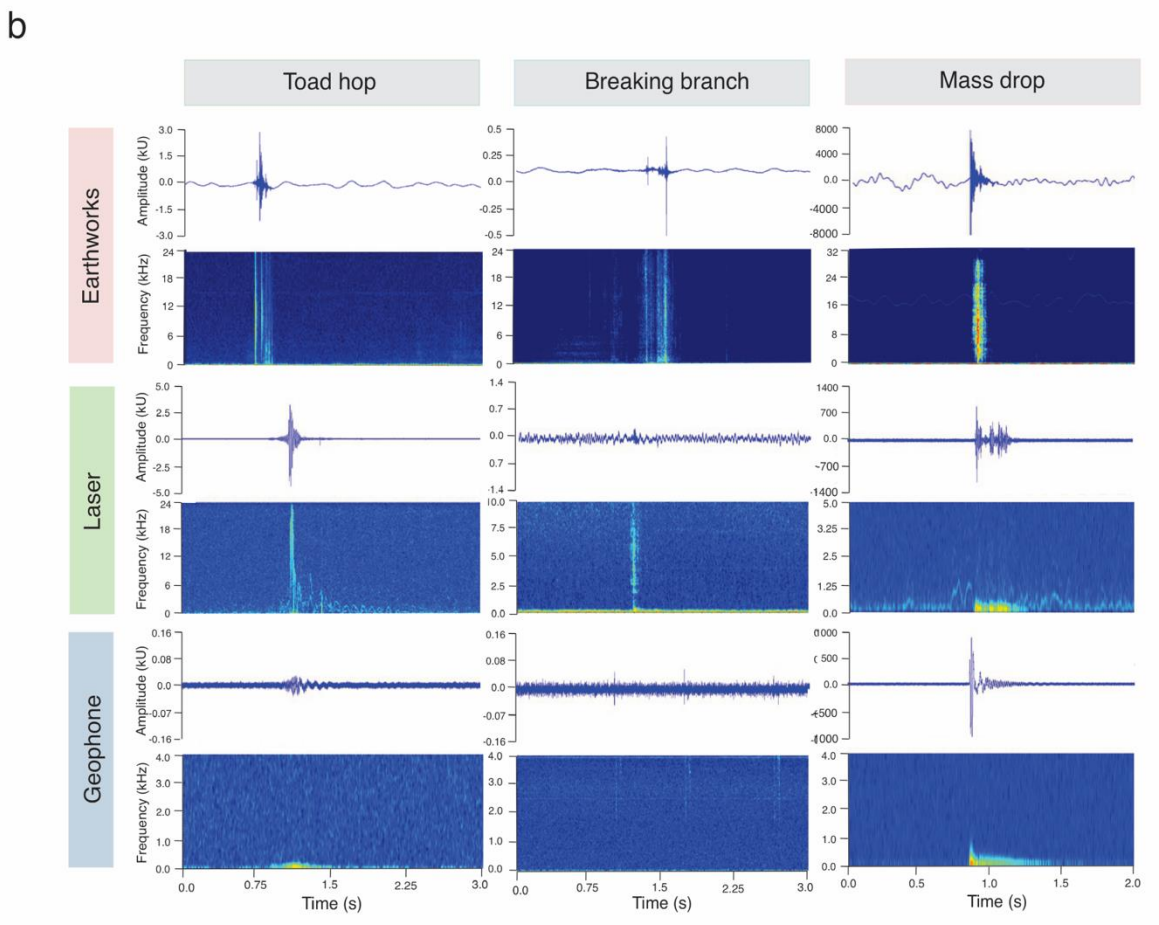

Figure 2.9. Extended waveforms and spectrograms for representative recordings of predator sounds and vibrations. b) Sounds and vibrations from live recordings of dropping masses and toad predators and playbacks of breaking branch sounds.

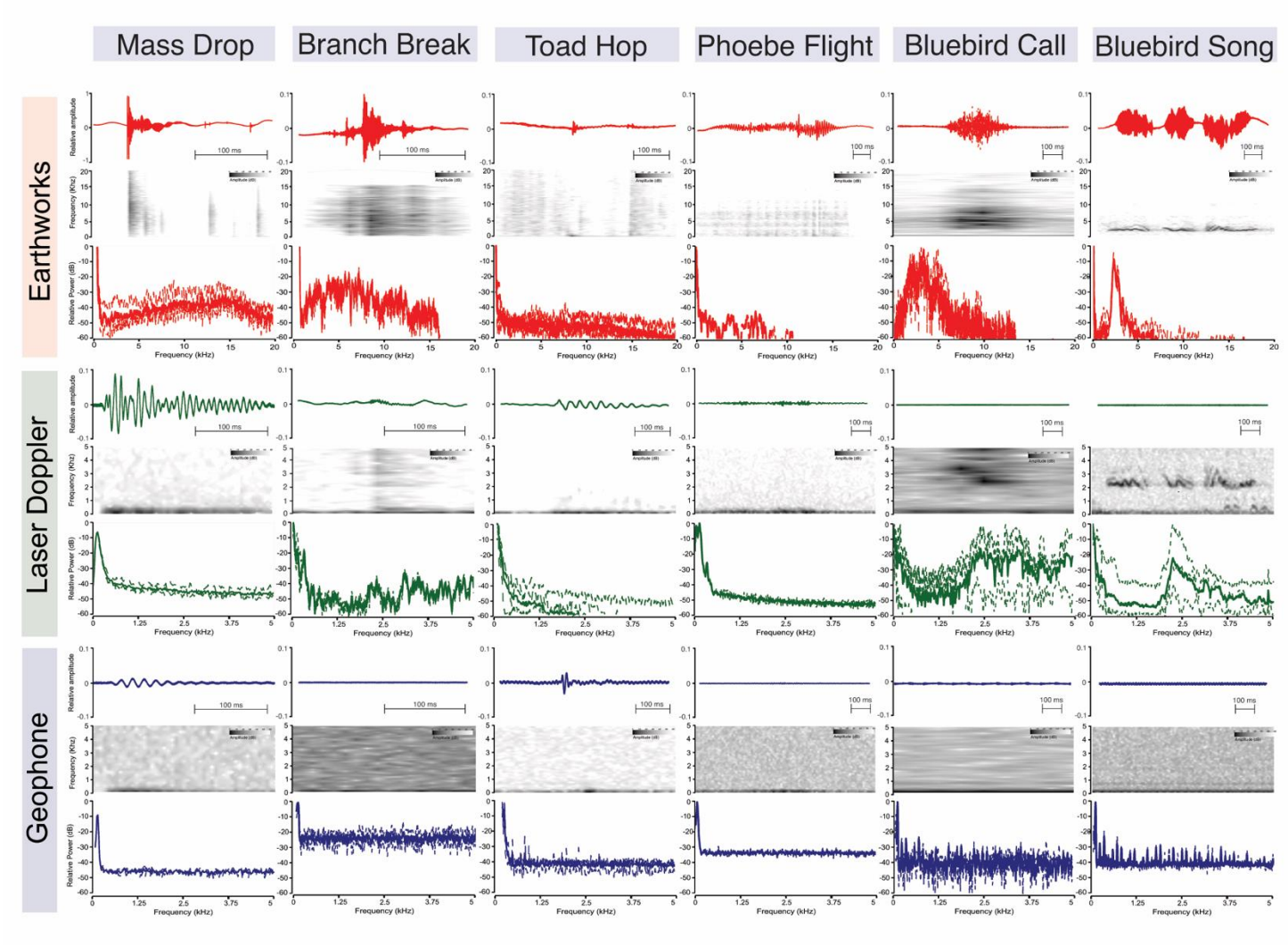

Figure 2.10. Waveforms, spectrograms, and power spectra for all predator stimuli recorded in the lab. Toad hops and mass drops were recorded live. All other stimuli were recorded from playbacks of pre-recorded stimuli. All spectrograms and power spectra were produced using a 4096-pt FFT (Hann window, 50% overlap).

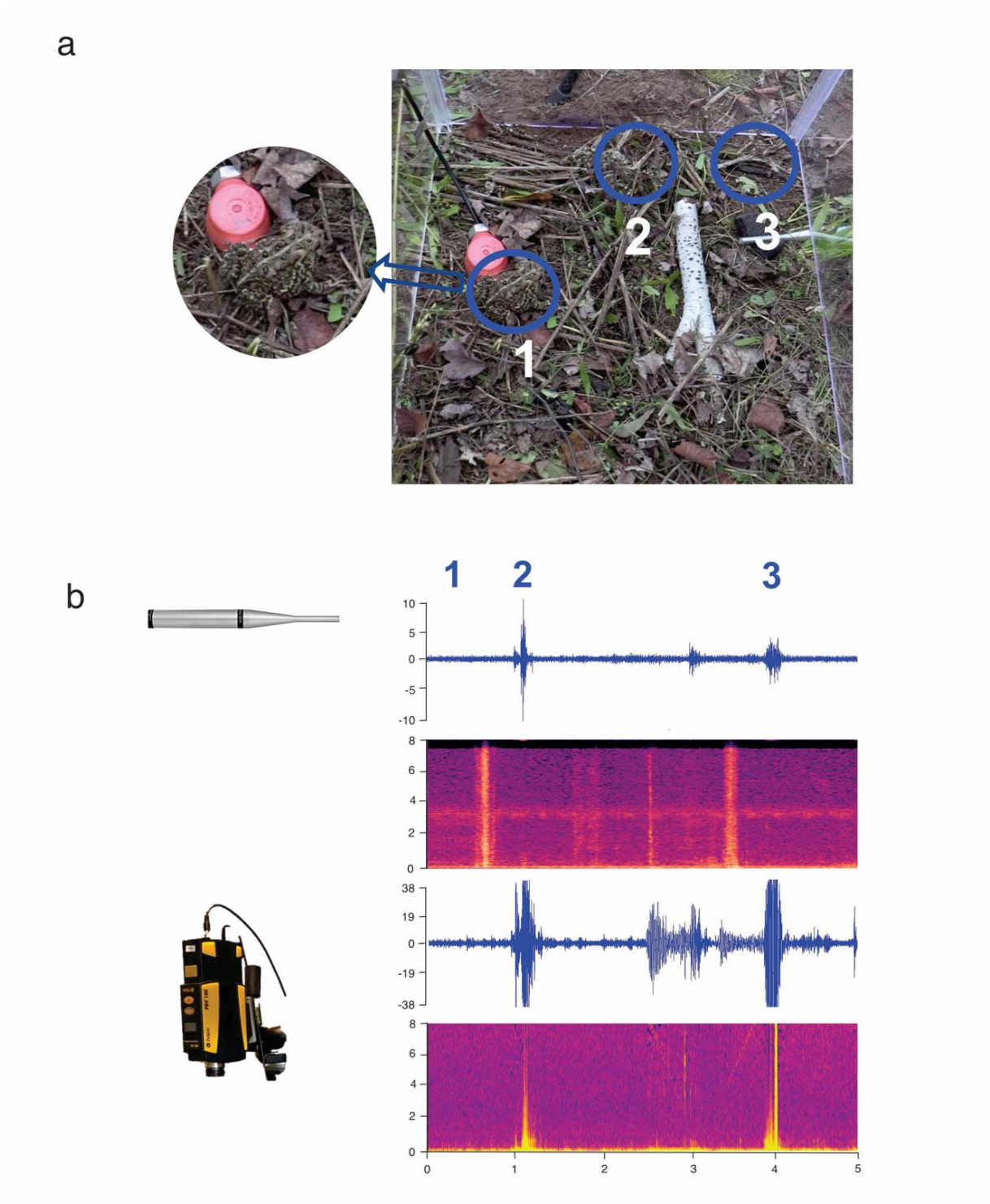

Figure 2.11. a) Diagram of field recording set-up for the represented field recording. Numbers denote where in the recording arena the recorded events occurred. b) Waveforms and spectrograms for recorded sounds and vibrations in the field on a microphone (top) and laser vibrometer (bottom).

How do recorded sounds overlap with cricket hearing sensitivity?

All predator stimuli recorded with the Earthworks microphone had some sound energy that overlapped with best sensitivity of the field *G. pennsylvanicus* tympanal organ, which is around 4-5 kHz (Figure 2.12). Incidental predator cues (e.g., toad hopping, breaking branches, bird flight) produced sounds that extended over a wide range of frequencies, allowing for the highest possibility of overlapping with cricket sensitivity (Figure 2.12). Toad hopping produced low frequency peaks that were highly variable depending on the part of the substrate the toad was moving on (first peak frequency: 893.55 Hz ± 1287.97 Hz, left bandwidth= 117.62 Hz ± 56.07, right bandwidth = 98.97 Hz ± 49.65). Most toads produced very low frequency first peaks (typically ~200 Hz), although hopping on leaf litter produced peaks between 1000-2000 Hz. While toads did not produce sounds with peak frequencies as high as 4000-5000 Hz, most of the recorded sounds still had energy extending up to 5000 Hz, overlapping with tympanal organ sensitivity in *G. pennsylvanicus* (Figure 2.12). Incidental sounds of bird flight were also very low frequency, with first peak frequencies at 70.53 Hz ± 0.185 and second peaks at 136.7 Hz ± 27.02. Both first and second peak frequencies were much lower than peak sensitivity of the tympanal organ, however there was still significant energy extending up to around 8 kHz in bird flight recordings, allowing for some overlap with tympanal organ sensitivity, particularly if the bird was at close range (Figure 2.12). Conversely, sounds produced by breaking branches had much higher peak frequencies (first peak frequency: 5976.80 Hz ± 862.48, n=3). First peak frequencies did not overlap directly with tympanal organ sensitivity, but there was substantial energy extending into

peak sensitivity of the tympanal organ of *G. pennsylvanicus*, suggesting that the tympanal organ can detect these sounds (Figure 2.12).

Bluebird calls and songs had distinct peaks that overlapped with sensitivity of the tympanal organ in *G. pennsylvanicus*. Eastern bluebird calls had first and second peak frequencies ranging between 3500-4000 Hz in all recordings (Table 2.2, Figure 2.6, 2.9). While this is near peak sensitivity of the tympanal organ, all recordings also had significant energy ranging from 2000 Hz through 6000 Hz, meaning that there was direct overlap with peak sensitivity of the tympanal organ (Fig. 2.12). Bluebird songs, conversely, had more concentrated energy around the peak frequency of the song (around 2 kHz), with energy only extending through about 4 kHz (Table 2.2, Figure 2.6, 2.9, 2.12). While there was still some overlap with peak sensitivity of the tympanal organ, it was much less compared to bluebird calls.

How do recorded vibrations overlap with cricket vibratory sensitivity?

Peak frequencies for the recorded vibratory stimuli were generally lower than the peak sensitivity of the subgenual organ in field crickets (Figure 2.13). Incidental vibrations from toad hopping recorded using the laser vibrometer and geophone produced very low frequency, broadband vibrations with first and second peak frequencies 100 Hz, whereas the field cricket subgenual organ is most sensitive to vibrations at frequencies between 500 Hz and 1 kHz. There was some energy extending through to about 1.5 kHz in laser recordings, suggesting that there was still overlap with subgenual organ sensitivity despite the peaks not directly overlapping. Vibrations produced by playbacks of breaking branches and Eastern phoebe flight were

only detectable by the laser, but not the geophone. Laser recordings of playbacks of breaking branches and phoebe flight also had first and second peak frequencies much lower than 500 Hz (Table 2.2, Figures 2.6, 2.9). Vibrations generated by playbacks of Eastern bluebird calls and songs were detected only by the laser vibrometer, not the geophone. Laser recordings of bluebird calls had peak frequencies that were either well below 500 Hz or that extended above the range detectable by vibratory sensory organs (Figures 2.6, 2.9, 2.13). Bluebird call vibrations however generally had some energy within the 500-1000 Hz range the subgenual organ is most sensitive to (Figure 2.13). Laser recordings of bluebird songs had virtually no overlap with sensitivity of the subgenual organ (Figure 2.13).

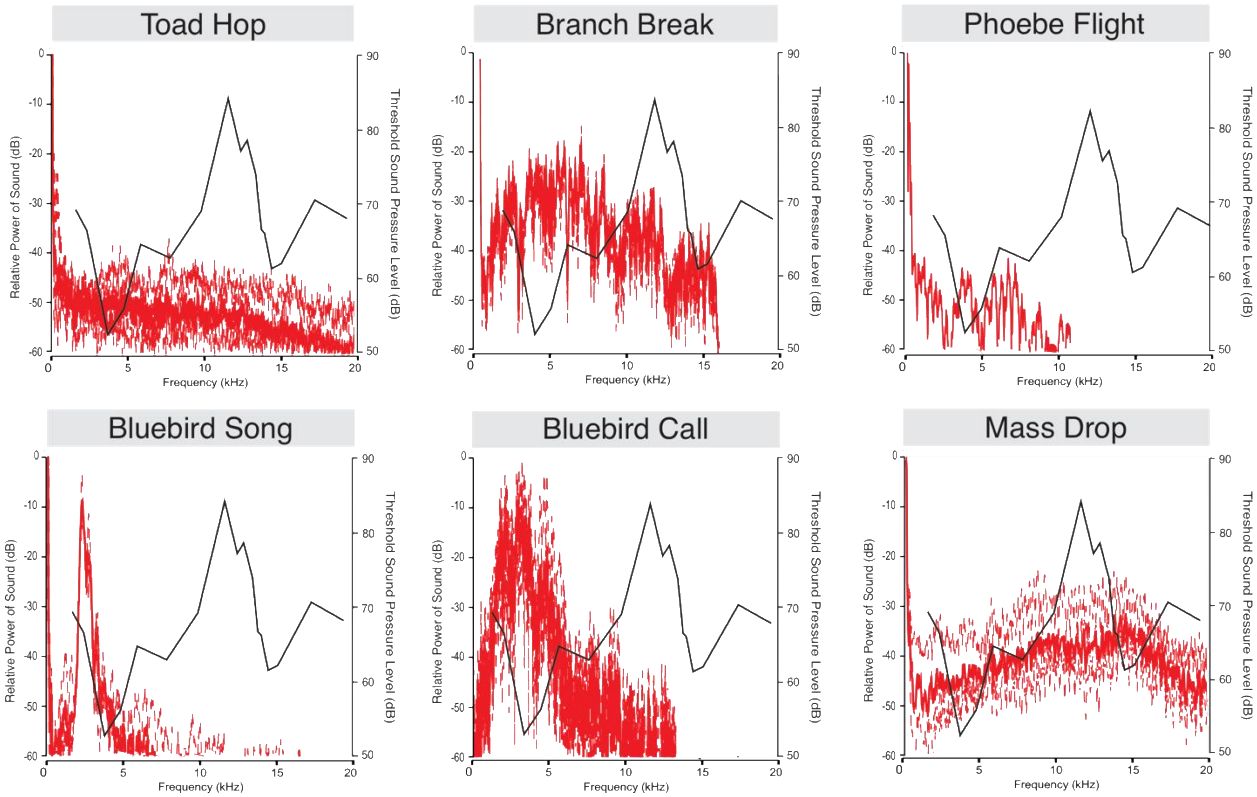

Figure 2.12. Power spectra for airborne sounds of predators recorded with earthworks microphone compared to sensitivity of the tympanal organ in *Gryllus* spp.. The shown tuning curve is reproduced from a whole nerve recording from *Gryllus campestris* (Nocke *et al.* 1972).

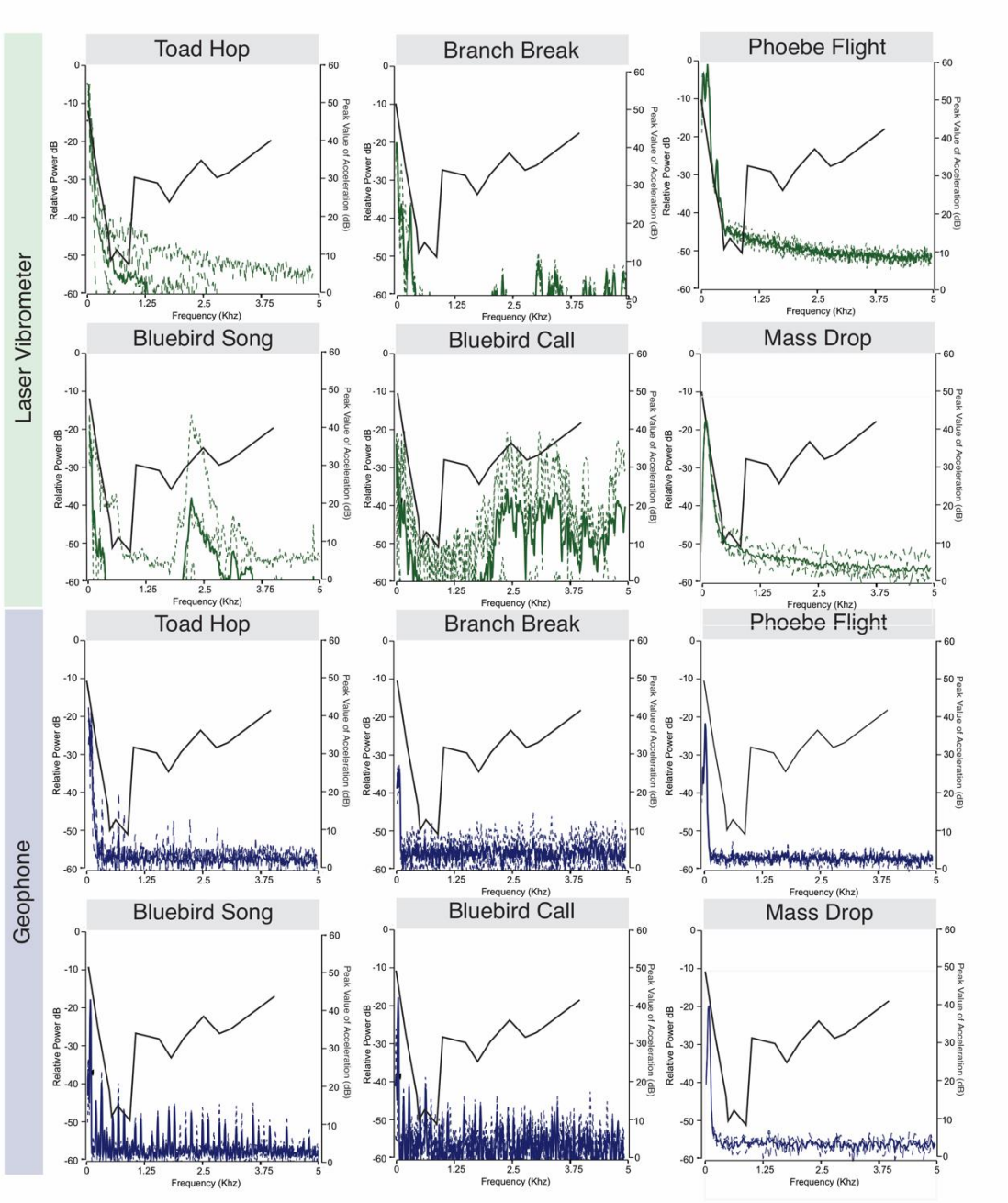

Figure 2.13. Power spectra for solid-borne vibrations generated by live recorded stimuli (Mass Drop, Toad Hop) and playbacks of pre-recorded stimuli compared to vibrational sensitivity of the subgenual organ in *Gryllus campestris*. Vibrations shown as recorded by the laser vibrometer (top) and geophone (bottom). The used tuning curve is reproduced from a whole nerve recording in *Gryllus campestris* (Nocke 1972).

Summary

All predator sounds characterized in this chapter have at least partial overlap with best sensitivity of the tympanal organ in *G. pennsylvanicus*. Of the characterized sounds, bluebird calls, breaking branches, and bluebird songs had the most direct overlap with best sensitivity of the tympanal organ. This suggests that these sounds are the most likely to be detectable by crickets, and therefore are likely to be relevant for risk assessment. While other predator sounds may not directly overlap with best sensitivity of the tympanal organ, it is probable that some are still detectable by crickets. For example, phoebe flight sounds were very low frequency, however there were frequencies in flight sounds that overlapped with both sensitivity of low frequency detecting units of the cricket acoustic sensory complex and some energy that overlapped with best sensitivity of the tympanal organ. It is likely, then, that crickets are still able to detect phoebe flight sounds. Toad hopping sounds also overlapped with sensitivity of the low frequency detecting units in the cricket ear; however, they were low amplitude and less likely to be detected by crickets in noisy environments.

Most predator vibrations characterized in this chapter did not overlap with best sensitivity of the subgenual organ in *G. pennsylvanicus*. Only laser recordings of playbacks of phoebe flight and breaking branches had direct overlap with sensitivity of the subgenual organ, but these vibrations were very low amplitude and potentially not accessible to crickets. While toad predator vibrations were high in amplitude compared to other vibration recordings, they were much lower frequency than the range the subgenual organ is most sensitive to. As vibrations did not generally overlap with sensitivity of the subgenual organ, my results suggest that the predator sounds

recorded in this chapter of my thesis are more likely to be relevant for risk assessment than recorded vibrations. It's possible, though, that on different substrates these vibrations may be detected by the cricket subgenual organ.

Discussion

Overview

In this chapter of my thesis, my goals were: 1) To characterize sounds and vibrations produced by known or proposed predators of *G. pennsylvanicus*; and 2) To compare the acoustic characteristics of these sounds and vibrations to sensitivity of the acoustic sensory organs in *Gryllus* spp. to make inferences on which sounds and vibrations may be available (i.e., detectable) to crickets for risk assessment. Characterized sounds included pre-recorded Eastern bluebird (*Sialis sialis*) calls and songs, Eastern phoebe (*Sayornis phoebe*) flight sounds, and breaking branches; as well as recordings of live American toads (*Anaxyrus americanus*) as they were hopping and capturing crickets. Characterized vibrations included vibrations transferred to the substrate from sound playbacks of Eastern bluebird calls and songs, Eastern phoebe flight and breaking branches; and live recorded vibrations from the American toad hopping. Two different vibration recording instruments were used to assess the efficacy of vibration recording instruments. This research approach is novel for the following reasons: 1. It was the first to assess the acoustic features of potential (non-bat) cricket predators; 2. It was the first to assess how potentially relevant airborne sounds from the environment (e.g. bird calls) might be transferred to a solid substrate.

Generally, the results showed that most of the sounds and vibrations had some overlap with acoustic sensitivity of *Gryllus* spp. based on spectral overlap. However, some of these overlapped more with the regions of best sensitivity, and these included airborne sounds of bluebird calls and breaking branches, and solid-borne vibrations of the toad hop. As my results showed potential overlap, playbacks of sounds to live crickets will be tested in Chapter 3 of this thesis. During my experiments, I also serendipitously recorded noteworthy sounds and vibrations of toads responding to being handled. Here, I will discuss my results based on each stimulus source, then summarize by providing recommendations for future studies.

Toad prey capture

Toads are ambush predators that largely consume terrestrial invertebrates, including many species of insect. They hunt insect prey by sitting and waiting for mobile prey to move nearby before launching an attack. To avoid being attacked by ambush predators such as toads, insect prey may use a variety of senses to detect the predator. For example, an insect may potentially detect a nearby predator before it attacks by picking up on scent cues, detecting the predator visually, or eavesdropping on incidental sounds and vibrations while the predator moves through the environment. My results provide the first experimental evidence that toads produce sounds and vibrations while stalking and capturing an insect prey that an insect may be able to use for predator avoidance.

My results show that toads generate airborne sounds (as recorded with the earthworks microphone) while hopping or crawling through the substrate and

approaching cricket prey. These sound amplitudes were generally very low relative to background in lab recordings. Lab recordings of incidental sounds produced by toad movement were broadband with low first peak frequencies that varied depending on the type of substrate the toad was moving on. While the peak frequencies of these sounds were low, the power spectra had energy extending throughout the whole 0-24 kHz range measured, with higher frequencies occurring especially when the toad was moving through leaf litter. While I could not compare amplitude characteristics between lab and field recordings of toads due to differences in equipment, the spectral characteristics of toad hopping sounds recorded in the field were comparable to those recorded in the lab. To my knowledge there are no studies characterizing the sounds produced by foraging toads, however similar sounds of animals moving through substrates have been previously described (e.g., Fullard 1988, Bernal *et al.* 2007, Goerlitz *et al.* 2008, Jacobs *et al.* 2008, Haff and Magrath 2010). While the peak frequency of recordings of animal movement through vegetation vary considerably depending on the substrate (e.g., rustling leaves vs. rustling grass), such sounds have generally been described as broadband with peak frequencies below 10 kHz (e.g., Fullard 1988, Goerlitz *et al.* 2008, Jacobs *et al.* 2008). While my recordings had low peak frequencies, they were similarly broadband and overlapped with best sensitivity of tympanal hearing in *Gryllus* spp. It is interesting to note that toad hopping sounds were incredibly low in amplitude, meaning they can be easily masked by background noise. It is possible that these sounds are 'intentionally' quiet to avoid being detected by prey. Ambush predators generally have much lower attack rates than pursuit predators, meaning that unsuccessful captures are costly (MacArthur and Pianka 1966). As such,

ambush predators have evolved a variety of strategies to avoid initial detection by prey. One such strategy is moving silently, as seen in some flying vertebrates (see Clark *et al.* 2020). While incidental air-borne sound cues produced by toad movement were very quiet, it is still possible that crickets may be able to detect them in quiet environments. This suggests that toad hopping sounds are a cue that crickets may use to detect predators, and I will be testing this hypothesis in Chapter 3 with playback experiments.

My results show that solid-borne vibrations were generated by hopping and crawling as toads approached cricket prey or moved through the substrate. These vibrations were relatively high in amplitude compared to background and had low peak frequencies (<100 Hz). Amplitude of toad hopping vibrations was not significantly affected by toad mass. The spectral characteristics of laser recordings of toad hopping performed in the field were comparable to those recorded in the lab, suggesting that recordings performed in the lab are comparable to what a cricket may experience in the natural environment. Therefore, such vibrations are potentially available to insect prey. While many insect prey species have been shown to use vibrations to detect and avoid predators, these examples are mostly limited to plant or leaf-borne vibrations (e.g., Castellanos and Barbosa 2006, Gish *et al.* 2011, Guedes *et al.* 2012, Gish *et al.* 2021). Solid-borne vibrations transmitted through soil or leaf litter have also been suggested to be relevant for predator detection and risk assessment in ground-dwelling insects. For example, some species of field cricket are suggested to respond to substrate vibrations by freezing, however these studies did not control for potential effects of airborne sound or visual stimuli in their behavioural assays (Hedrick 2000, Adamo *et al.* 2013). For example, in one study behavioural responses were measured in response to a human

researcher tapping on the enclosure (Hedrick 2000). In the other study, behavioural responses were measured when crickets were exposed to a robotic toy hamster producing vibrations (Adamo *et al.* 2013). In both cases, it is likely that there is an airborne sound component being generated as well as a visual stimulus that crickets may respond to.

It is likely that if vibrations produced by field cricket predators overlap with sensitivity of their vibratory sense organs, they can be used to detect and avoid predators. My results showed that toad movement sometimes produced vibrations that overlapped with sensitivity of the subgenual organ in *Gryllus spp.*, however there was considerable variation between recordings. This is likely due to heterogeneity of the substrate in the recording arena. Substrate type is known to influence the acoustic characteristics of vibrations, as each substrate has its own attenuation and filtering properties (reviewed by Elias and Mason 2014). It is likely then that differences in vibrational frequencies produced by toad cues are at least partially related to the part of the substrate the toad was hopping on, and crickets may only be able to detect vibrations from oncoming toad predators on specific substrates. It is certainly possible that crickets may use vibrational cues to detect toad predators, however this will not be tested in Chapter 3 as the latter portion of my thesis will focus solely on sound playbacks.

Toad release calls and vibrations

In addition to the incidental sounds and vibrations generated by hopping while pursuing and capturing cricket prey, American toads produced two types of acoustic

signals during lab experiments: an airborne chirp recorded by the microphone and a solid borne vibratory signal recorded with the laser and geophone. Based on the literature, I have called these signals “release calls” and “release vibrations” (Sullivan 1992). While these signals are not directly related to the topic of my thesis, they are of interest. Both the release call and release vibrations have been noted in the literature in mating contexts, however only the vocalizations have been characterized. Below, I provide a brief discussion of each signal, how they differ, and review their known and proposed functions.

In my experiments, toads produced airborne chirps only while being handled when being placed in or removed from the terrarium, and particularly so when light pressure was applied to the sides of their abdomen. Since these signals could potentially be indicators of stress, I only recorded release signals at the end of a live recording session with the toads to minimize the effect of stress on hunting behaviour. As such, airborne sound and vibrations were recorded separately as they were in live recordings of toad hunting, and only airborne sound was recorded from release calls. I recorded this call from both male and female toads, but more males produced the call than females. Many species of toad, including the American toad (*Anaxyrus americanus*), have been documented to make similar calls to those recorded in this chapter (Schmidt 1972, Sullivan 1992, Leary 1999). These sounds, termed “release calls”, generally are emitted during amplexus. In males, they are emitted when mounted by another male to stimulate release, and in females they are emitted to stimulate release when unreceptive to mating (Sullivan 1992). My results showed that these chirps had peak frequencies of about 1.6 kHz, which is comparable to other

characterizations of release calls in *A. americanus* (Sullivan 1992). While these release calls have largely been documented in mating contexts, a few species of toad are known to produce similar sounds in antipredator contexts (Marchison and Anderson 1978). For example, some species of toad produce similar sounds when captured by snake predators, and the sounds have been proposed to be used to startle predators into releasing a captured toad (Marchison and Anderson 1978). It is possible that these release calls function in both mating and antipredator contexts to signal release, however more research is needed on possible functions of release calls outside of mating. For example, I would recommend recording release calls in a mating context as well as in a simulated predator attack to compare signals and assess if they are in fact the same signal.

Toads of both sexes also produced a vibrational signal while being handled during live recordings with the laser vibrometer and geophone while the toad was against the substrate. These vibrations are considered to be different and separate from the airborne chirps described above, although they were usually (but not always) produced simultaneously with release calls. This vibrational signal is proposed to function in tandem with release calls to initiate release when a toad is unreceptive to mating (Blair 1947, Schmidt 1972). Release vibrations have been mentioned in many toad species, including the American toad (e.g., Blair 1947, Schmidt 1972), but they have not yet been characterized. To my knowledge, documented antipredator behaviours exhibited by toads include crouching, remaining immobile in the presence of a predator, hiding, body inflation, chin tucking, and defense vocalizations (Marchison and Anderson 1978, Heinen 1994). However, there is no research investigating if toads

produce vibrational signals when captured by predators. Given that toads generally produced release vibrations simultaneously with release calls, and release calls have been documented in antipredator contexts, it is possible that this vibration may also act as a defense signal. Such vibrations may be more effective when directed at predator species with less sensitive hearing but more sensitive vibratory senses. For example, snakes rely heavily on detecting vibrations through their jawbone (Christensen *et al.* 2012), so it is possible that vibrations would function as a defense mechanism when captured by a snake. I propose that release calls and vibrations may function in both mating and antipredator contexts, however more testing is required. For example, sounds and vibrations produced by toads in a simulated predator attack could be compared to the sounds and vibrations produced by unreceptive toads in mating contexts to assess if release calls and vibrations function in antipredator contexts.

Breaking Branches

As terrestrial predators approach prey, they may produce incidental sounds while moving through vegetation such as rustling leaves and grass or breaking twigs (Fullard 1988). In my thesis, I played back pre-recorded sounds from breaking branches over a loudspeaker and measured acoustic characteristics of these sounds. Airborne sounds produced by breaking branches had first peak frequencies of about 6 kHz, however energy from these sounds was not highly concentrated around the peak. As such, significant energy was present from 2-12 kHz on power spectra from breaking branch sounds. Other studies that have characterized sounds produced from breaking twigs or branches have found similar results, with energy from these sounds in frequencies ranging from <5 kHz well into the ultrasonic range (Fullard 1988). Since sounds

produced by breaking branches contain a broad range of frequencies, there is substantial overlap with sensitivity of the tympanal organ in *G. pennsylvanicus*. This suggests that crickets are potentially capable of detecting these sounds. My results showed that playbacks of airborne sounds produced by breaking branches did not transfer vibrations to the substrate. It is important to note that although playbacks of breaking branch sounds did not generate any substantial solid-borne vibrations, it is likely that the act of breaking a branch would generate vibrations in the substrate. To my knowledge such vibrations have not been characterized, but it is likely that such cues could be important for predator detection in insects.

Bird Flight & Vocalizations

Insects are prey to a variety of insectivorous bird species. There are a few strategies that bird predators may use to catch insect prey, including aerial hawking (i.e., catching and pursuing flying insects while the bird is in flight), gleaning an insect off a surface while the bird is in flight (i.e. “sally-gleans” or “hover-gleans”), and catching stationary prey off a surface while the bird is standing or hopping (i.e., “perch-gleans”) (Fitzpatrick 1980, Robinson and Holmes 1982). Bird predator species produce a variety of acoustic signals and cues that insect prey may use for predator detection and risk assessment. These include signals such as conspecific communication calls and songs, or incidental cues produced by foraging such as rustling vegetation and flight sounds. Moreover, the transfer of sounds produced by bird predators to the substrate may also provide relevant cues for predator detection. Here, I review the features of such sounds and vibrations for bird flight, bird songs, and bird calls. I describe the overlap between

the sounds and cricket hearing, and also describe how these sounds may or may not be transferred to the substrate and if so, how they overlap with cricket vibration sensitivity.

Phoebe flight

Bird flight produces airborne sound through beating of the wings that may be a relevant cue for risk assessment in insects. My results showed that first peak frequencies of Eastern phoebe flight were very low (<100 Hz) but had significant energy extending up to about 5 kHz. While other studies have reported slightly higher peak frequencies of bird flight sounds, the sounds were still quite low frequency (~1 kHz) (Jacobs *et al.* 2008, Fournier *et al.* 2013). Bird flight has been suggested to be an important cue for predator detection by insect prey, however this research has been focused on flying insects (Jacobs *et al.* 2008, Fournier *et al.* 2013). For example, some species of moth are known to respond neurally to playbacks of bird flight sounds and it has been suggested that some moths respond behaviourally to sounds generated by foraging birds by fleeing (Jacobs *et al.* 2008, Fournier *et al.* 2013). However, these sounds may also be used by terrestrial insects such as field crickets to assess risk of predation. Recordings of Eastern phoebe flight had a small degree of overlap with peak sensitivity of the tympanal organ of *G. pennsylvanicus*. While it is possible that the tympanal organ of *G. pennsylvanicus* can detect some of the higher frequencies produced by bird flight, it also seems likely that these sounds could be detected by low frequency detecting units in the acoustic sensory system. The subgenual organ for example is reported to detect low frequency airborne sound in addition to vibrations in some other species of field cricket (Nocke 1972, Esch *et al.* 1980), with broadly tuned

sensitivity between 800 Hz and 2 kHz for airborne sound in *Gryllus campestris* (Nocke 1972). It is possible, then, that sensitivity of both the tympanal ear and subgenual organ overlap with bird flight sounds. Based on these results, I will be testing if crickets are using bird flight sounds for risk assessment using playback experiments in Chapter 3.

Some airborne sounds may be transmitted to the substrate as vibrations. To my knowledge, there is no evidence of insects using such vibrations in the context of risk assessment. However, these cues may still be relevant to insect prey for predator detection and evasion. My results showed that playbacks of phoebe flight sounds produce some solid-borne vibrations detectable by the laser vibrometer. These vibrations were generally low amplitude and low frequency, as they were recorded from a solid piece of wood on the substrate. Nonetheless, there were some peaks that overlapped with the sensitivity peak at around 500 Hz. It is possible, then, that crickets may detect these vibrations as the bird flies closer, particularly if the cricket is on a more lightweight substrate such as leaf litter. I would recommend that vibrations generated from playbacks of bird flight be recorded on a variety of substrates (e.g., soil, leaf litter, grass) to investigate if these vibrations may be more useful in other environments. Moreover, substrate-borne vibrations generated from bird flight may also be recorded live as birds glean insect prey off surfaces to investigate if these vibrations are generated in an insect's natural environment to determine if this cue is available to insect prey.

Bird Vocalizations

Predator communication signals can be a valuable source of information for prey to assess risk. Eavesdropping on communication signals is widespread amongst the animal kingdom, and predator communication signals are known to be important for risk assessment in both vertebrate and invertebrate prey (reviewed in Yack *et al.* 2020). While the focus of such studies in invertebrate prey has been on eavesdropping on bat echolocation signals, it has been proposed that insects also eavesdrop on other predators' communication signals, including avian predators. For example, it has been shown that *Morpho peleides* is capable of detecting avian predator vocalizations (Mikhail *et al.* 2018), and a few butterfly species of the genus *Erebia* are known to respond behaviourally to frequencies corresponding with bird communication signals (Ribaric and Gogola 1996). It is also likely that other insects with broadly tuned ears may be capable of detecting and eavesdropping on communication signals produced by their avian predators for risk assessment. Here, I tested two vocalizations from the Eastern bluebird (*Sialis sialis*), an insectivorous bird known to hunt terrestrial insects including field crickets, to investigate if fall field crickets may be able to eavesdrop on their communication signals for risk assessment.

Eastern bluebird song

The first bird vocalization I tested in this chapter was a song produced by the Eastern bluebird. In many bird species, including the Eastern bluebird (*Sialis sialis*), songs are longer vocalizations produced most often by males that function to defend territory and attract mates (Gowaty and Plissner 2020). Eastern bluebird songs are loud,

with sound pressure levels being reported to range from 40.33-87.93 dB at one metre from the vocalizing bird (Kight and Swaddle 2015). I played back Eastern bluebird songs to a microphone at sound pressure levels within the documented range for this species and measured their spectral characteristics. Peak frequencies of Eastern bluebird songs were around 2 kHz with energy concentrated around the peak, as would be expected for a communication signal. While the spectral characteristics of Eastern bluebird songs do not directly overlap with best sensitivity of the tympanal organ in *G. pennsylvanicus* (~ 4 kHz), there is some overlap. Playbacks of bluebird songs did not produce any detectable substrate vibrations. Since Eastern bluebird songs do not directly overlap with best sensitivity of the cricket tympanal organ, I will not be testing if crickets respond behaviourally to these sounds in the playback experiments in Chapter 3. However, it is important to note that a lack of direct overlap with tympanal organ sensitivity does not necessarily mean that crickets cannot detect or respond to Eastern bluebird songs. Given the tonotopic arrangement of the crista acoustica in the cricket tympanal ear (Oldfield *et al.* 1986), it is very possible that a portion of scolopidia (acoustic sense cells) within the tympanum can respond to these frequencies. This could be tested using a playback experiment to neural preps of the cricket tympanal organ to investigate if the tympanum can detect these frequencies before testing for behavioural responses to these sounds. Similarly, it would be good to assess how bird calls are transferred as vibrations to different substrates in natural contexts.

Eastern bluebird call

In birds, calls tend to be shorter communication signals that are used equally by both sexes in a variety of contexts, including advertising the bird's location to conspecifics, warning others of a predator's presence, alerting their flock to a newly discovered food source, and begging calls used by young birds to alert their parents that they need to be fed. Calls may differ in acoustic characteristics depending on the context they are being used in. The Eastern bluebird call used in my thesis is the chatter call often used to alert conspecifics of ground predators or approaching humans, which tends to have sound pressure levels similar to the bluebird songs discussed above (Kight and Swaddle 2015, Gowaty and Plissner 2020). My results showed that Eastern bluebird calls played back at approximately the same dB reported in the literature had peak frequencies of about 3.5-4 kHz, meaning that it overlaps directly with the sensitivity range of *G. pennsylvanicus* hearing. Playbacks of bluebird calls did not produce any detectable substrate vibrations. Based on these results, I will be testing if crickets respond to sound playbacks of Eastern bluebird calls with antipredator behaviours in Chapter 3.

Recommendations regarding instrumentation

In my thesis, I used three devices to record stimuli produced by predators of *Gryllus pennsylvanicus*: 1) an Earthworks QTC50 microphone, 2) a laser Doppler vibrometer, and 3) a geophone. The Earthworks microphone recorded airborne sound, and the laser and geophone recorded substrate vibrations. The devices used for my thesis were chosen based on their specifications and were capable of recording

acoustic events across the range of frequencies cricket acoustic sensory organs are sensitive to. The laser was highly sensitive to surface level vibrations and was sufficient for detection of all vibrations produced by *Gryllus* predators. It also was capable of detecting higher frequency vibrations and even sounds that transferred vibrations to the surface of the substrate. For example, in Figure 2.10, it is apparent that the laser is detecting airborne sound from bird calls and bird songs. As such, the laser is good for detecting surface vibrations from lightweight objects such as leaves and lightweight sticks and twigs. The geophone comparatively was better suited for recordings of lower frequency vibrations that propagated deeper into the substrate. For example, the geophone detected vibrations produced by the dropping mass and by toad hopping, but did not pick up any vibrations from bird flight playbacks, unlike the laser.

Summary and Future Directions

Many of the sounds recorded in Chapter 2 overlapped at least partially with sensitivity of the acoustic sensory organs in *G. pennsylvanicus*, however virtually none of the recorded vibrations had direct overlap with the dominant frequency of 4 kHz. Of the recorded sounds, bluebird calls overlapped the most with best sensitivity of the tympanal organ. While the other sounds did not have peak frequencies that directly overlapped with tympanal organ sensitivity, all recorded sounds had at least some energy within the range where the tympanal organ is most sensitive. It is possible that other cells within the tympanal organ may be capable of detecting higher or lower frequency sounds outside of the 4 kHz peak sensitivity, which is based on a whole nerve branch recording of all cells. Vibrations comparatively had little overlap with the

proposed sensitivity of the subgenual organ. This could be due to the natural resonance the substrate used for recordings. In field settings with different types of substrate, it is possible that these vibration frequencies and amplitude levels may overlap more directly with sensitivity of the subgenual organ. It is also possible that there are some cells in the subgenual organ that respond to these frequencies outside of sensitivity shown in tuning curves of the subgenual organ in *Gryllus* spp. Further research is needed to better understand which predator sounds and vibrations are accessible to crickets for risk assessment. For example, it would be worthwhile to characterize sounds and vibrations produced by more predator and non-predator species to determine if they may be detectable by crickets. Examples of such sounds include rustling leaves, sounds and vibrations produced by foraging of other predator species, and alarm signals produced by non-predator species. It would also be meaningful to perform recordings of these sounds and vibrations in a natural environment to determine if they are accessible under natural conditions as well as laboratory conditions. To assess if crickets are attending to sounds and vibrations that overlap with sensitivity of their acoustic sensory organs and are potentially useful for risk assessment, playback experiments need to be performed. In Chapter 3 of my thesis, I perform some preliminary playback experiments to live crickets.

Chapter 3: Playback of acoustic cues (Experiment 2)

Introduction

Insects are attacked and consumed by numerous vertebrate and invertebrate enemies, and consequently have evolved many adaptations, including sensory specializations, for detecting danger and assessing risk (Stumpner and von Helversen 2001, Gilliot 2005). At present, evidence for acoustically-mediated predator detection and risk assessment in insect prey is limited (Yack *et al.* 2020). Here, I present a short overview of how acoustic information is used in risk assessment by insect prey based on the type of acoustic information transmitted (see also Chapter 1). Solid borne vibrations are generated by propagating vibrations through a solid medium and are detected by the subgenual organs, trichoid sensilla, and campaniform sensilla in insects (Yack 2004). In the context of risk assessment, insects are known to use stem and leaf vibrations generated by approaching insect predators (e.g., Hill 2009, Guedes *et al.* 2012, Gish 2021). However, little attention has been given to the role of vibrations produced by other predators including mammalian, reptilian, and amphibian predators in the context of risk assessment. Airborne vibrations (sounds) can be transmitted as either near-field or far-field sound. Near-field sound is transmitted over short distances by displacement of air molecules close to the sound source and is detected by some insects by light weight-structures such as trichoid sensilla, or antennae (via Johnston's organs) (Yack 2004). The role of near-field hearing in risk assessment is poorly understood, however it is thought to be important for the detection of aerial insect predators and parasitoids (Yack *et al.* 2020). For example, caterpillars are known to detect and flick in response to near-field sounds comparable to flight sounds produced by their insect predators and parasitoids (e.g., Taylor and Yack 2019). Far-field sound is

transmitted over long distances by airborne pressure waves and is usually detected by the tympanal organ in insects (Yack *et al.* 2004). In the context of risk assessment, tympanal hearing has been studied extensively for the detection of bat predators by flying insects (e.g., Hoy 1992, Miller and Surlykke 2001, Yager 2012, Pollack 2015, Pollack *et al.* 2016). However, other predators that consume insect prey, such as birds, lizards, toads, and dragonflies, may also produce sounds that conceivably could be used by insect prey for detection and risk assessment. Such predator-generated sounds could include communication signals (e.g., calls and songs that they use for communicating with conspecifics) as well as sounds incidentally generated by body movements, such as crawling or flight. For example, there is evidence that some insects can detect sounds produced by bird flight, and these sounds are proposed to function in risk assessment by prey insects (Jacobs *et al.* 2008, Fournier *et al.* 2013). Yet, there are few studies investigating whether predator sounds (other than bat echolocation signals) are used by insect prey for risk assessment, and experiments are necessary to test how insects might respond behaviourally to these stimuli. In this thesis I tested this using the fall field cricket, *Gryllus pennsylvanicus*, as a model.

Field crickets of the genus *Gryllus* have been studied extensively for their acoustic mating systems and rely on tympanal hearing to find mates and defend their territories (Hedwig 2006, Jang *et al.* 2008, Stevenson *et al*. 2013, Schöneich 2020). Some flying species of field cricket are known to use tympanal hearing to detect and avoid bat predators by eavesdropping on their echolocation signals (e.g., Pollack and Martins 2007, Pollack 2015). However, field crickets are also prey to an array of predator species, including insectivorous birds, amphibians, reptiles, and small

mammals such as rodents (Hedrick 2000) (Table 2.1). It has been proposed that crickets may detect predators by their vibrations. For example, *Gryllus integer* males responded to vibrations from thumping on the outside of the experimental arena with increased latency to emerge from shelter and call cessation (Hedrick 2000). In another study conducted by Adamo *et al.* (2013), crickets of the species *Gryllus texensis* showed increased hiding behaviour in response to vibrations generated by a robotic mock predator. Neither of these studies, however, used sounds and vibrations produced by or resembling real cricket predators, and the latter did not separate the sounds and vibrations produced by the mock predator, so it cannot be concluded whether antipredator behaviours were in response to solid or airborne vibrations or a visual stimulus.

In Chapter 2 of this thesis, I tested the hypothesis that *G. pennsylvanicus* uses sounds and vibrations to assess risk. My results showed that non-bat predator species of *G. pennsylvanicus* produce sounds and vibrations, and that there is some overlap with sensitivity of their acoustic sensory organs. It appeared that bird communication signals are the most likely to overlap with sensitivity of the cricket tympanal organ, although all predator sounds had some energy overlapping with hearing sensitivity in *G. pennsylvanicus*. There may also be individual auditory cells within the tympanal organ that are more sensitive to specific frequencies that are not necessarily portrayed by the whole nerve tuning curve (Figure 2.1). It is possible, then, that many of the sounds characterized in Chapter 2 may be used for predator detection and risk assessment by fall field crickets.

The goal of this chapter (Chapter 3) is to further test the hypothesis that *G. pennsylvanicus* uses airborne sounds produced by natural enemies for risk assessment. I conduct some preliminary experiments to test behavioural responses of *G. pennsylvanicus* to airborne sounds of their predators. I test if crickets respond behaviourally to sound playbacks of toad hopping, Eastern phoebe flight, and Eastern bluebird calls. If fall field crickets are using airborne sounds to detect and avoid predators, I predict that they will respond with antipredator behaviours when presented with acoustic stimuli produced by their predators. I tested this prediction in a playback experiment in which I video recorded crickets while they were presented with a sound of a known or proposed predator. I then assessed if presentation of predator stimuli induced antipredator behaviours and if crickets responded differently to stimuli produced by different predator species. Table 2.1 in Chapter 2 provides a summary of documented antipredator responses in crickets. Antipredator responses assessed in this chapter include freezing, fleeing, and changes in mobility.

Methods

Study species

I used the fall field cricket, *G. pennsylvanicus*, as a model organism to investigate if predator acoustic stimuli elicit antipredator responses in insects. Fall field crickets were sourced from the same laboratory colony used in Chapter 2. The colony was housed in large plastic storage bins (dimensions: 61 x 41 x 32 cm) separated by life stage (i.e., separate bins for pinheads, juveniles, and adults). The colony was housed in a temperature-controlled room at the University of Ottawa at 25°C under a 12:12

light/dark cycle and provided shelter and ad libitum food and water. Crickets to be used for experiments were isolated from the colony prior to their final molt, identifiable by the formation of wing bugs in both sexes and the development of an immature ovipositor in females. Isolated crickets were placed inside of 19 L plastic buckets (dimensions: 35 cm diameter, 40 cm height) lined with brown paper towel to prevent crickets from slipping on the plastic. Each isolated cricket was given a piece of egg carton for shelter and food and water ad libitum. Crickets were housed in isolation until 48 hours after their final moult to account for any effects of the physiological cost of moulting on behaviour. All crickets used for experiments were visually inspected following their final moult to ensure that they were generally in good condition (e.g., no missing limbs or appendages and no visible damage to the exoskeleton). As experiments were conducted in the Yack Lab at Carleton University, crickets were transferred from the University of Ottawa to Carleton University and allowed 24 hours to acclimate before being used for experiments.

Experimental set-up and equipment

Figure 3.1 shows an overview of the set-up used for playback experiments. The set up included an arena (i.e. plastic bucket), a video camera, a sound speaker, a microphone and a data recorder. The experimental arena was a 19 L paint bucket (RONA Inc., Boucherville, QC, Canada, dimensions: 31 x 37 cm). To reduce the effects of external sounds and vibrations, playback experiments were performed in a sound-attenuated room (Eckel Industries Ltd., Cambridge, MA, USA) on an antivibration table (Figure 3.1). To record the playback experiments, a Sony handycam camcorder (hd-rxr520v, Sony, Tokyo, Japan), on a tripod, was positioned above the arena with a full

view of the bottom of the container (Figure 3.1). Sounds played back were recorded using the internal camera microphone. Sounds were played from a laptop computer connected to a Bose SoundLink Color II speaker (Bose Corporation, Framingham, Massachusetts, United States). Table 3.1 provides an overview of sounds used for playback experiments and their sources. The computer used to play sounds through the speaker was positioned out of the crickets' view so there were not any additional visual stimuli that may bias behavioural responses. The speaker was clamped to a stand and positioned 20 cm above the arena. Prior to beginning the experimental trials, each sound stimulus was re-recorded in the experimental set-up using an Earthworks QTC50 microphone (not shown in Figure 3.1) to allow comparison between the original recorded sound and the sound as it was presented during the playback. For these stimulus recordings, the speaker was positioned at the same height above the arena as it would be during the playbacks, and the microphone was positioned as close to the bottom of the arena as possible where the cricket would be positioned during playback trials. Sounds were recorded to a Fostex data recorder at with a sampling rate of 48 kHz, then exported to a laptop computer as .wav files. Loudness of all played back sounds was measured using a sound level meter (Type 2239, B&K, Nærum, Denmark) positioned beside the microphone in the plastic bucket. Supplementary Material C shows data for the loudness of all sounds played back to crickets.

Table 3.1. Overview of sounds used for playback experiments and their sources.

Sound	Predator species	Source	Number of exemplars
Bird call	Eastern bluebird (*Sialis sialis*)	Online	3
Bird flight sounds	Eastern phoebe (*Sayornis phoebe*)	Live recording	1
Toad hopping	American toad (*Anaxyrus americanus*)	Live recording	3
200 Hz pure tone	NA	Online	1
White noise	NA	Online	3

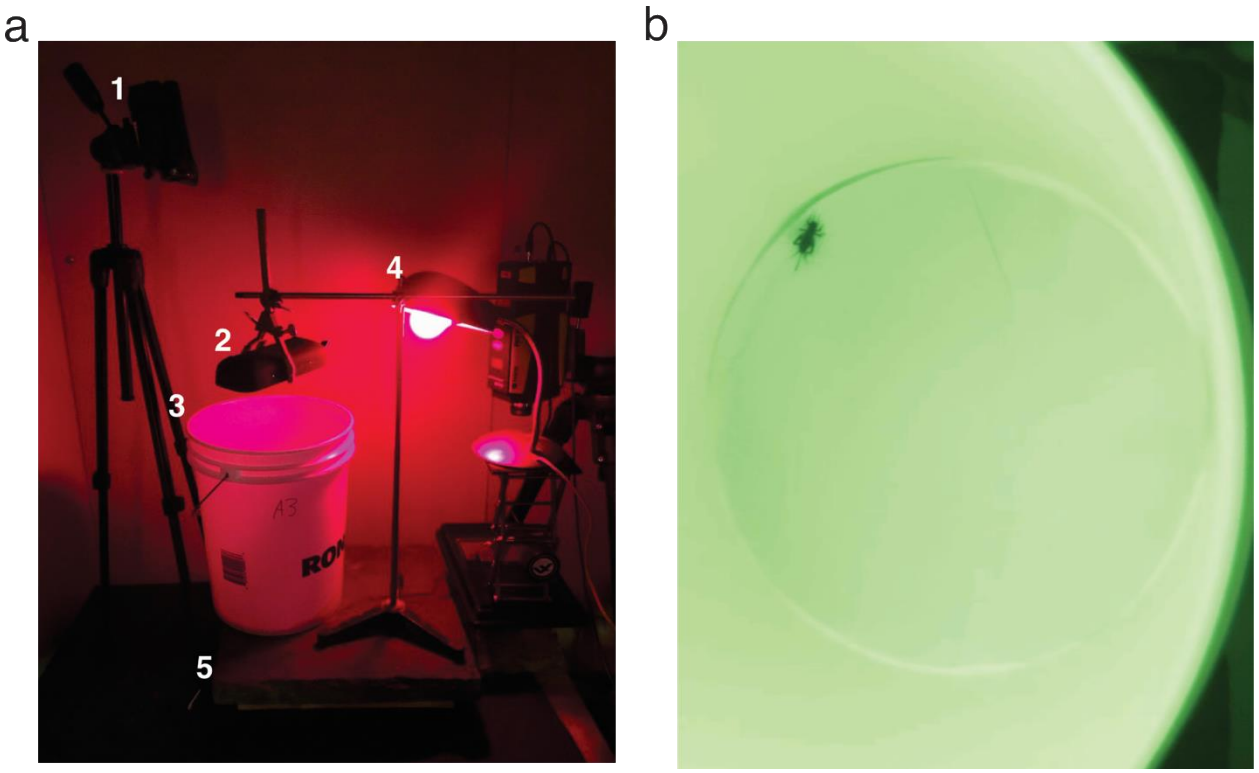

Figure 3.1. Set-up for predator sound playback experiments. a. Overview of experimental set-up. Shown are the following pieces of equipment: 1) camcorder, 2) speaker, 3) experimental arena (bucket), 4) red light, and 5) antivibration table. b. Top-down view of a cricket inside the bucket during playback experiments.

Playback experiment

A total of 50 trials were conducted, although six of these trials were discarded as the cricket did not move during the entirety of the playback. In each trial, I presented an

individual adult cricket with one of five different acoustic stimuli, repeated three times separated by one minute of silence. Each stimulus was between 1-2 seconds in duration. The five stimulus options included the following: 1) Eastern bluebird calls (*Sialis sialis*) (n=7 crickets, 3 males and 4 females); 2) Eastern phoebe (*Sayornis phoebe*) flight sounds (n=10 crickets, 5 males and 5 females); 3) Sounds produced by an American toad (*Anaxyrus americanus*) hopping in leaf litter (n=9 crickets, 5 males and 4 females); 4) a 200 Hz pure tone (n=9 crickets, 4 males and 5 females); or 5) white noise as a control (n=9 crickets, 3 males and 6 females). Figure 3.2 shows waveforms and spectrograms for all sounds used in the playback experiments. Each cricket was weighed using a digital balance following their trial (see Supplementary Material D for more information on crickets used). An overview of the procedure is described below.

Crickets used for playback trials were transferred from the lab they were reared in at the University of Ottawa to the Yack Lab at Carleton University 24 hours before being used for experiments. All crickets were isolated and transported in the same bucket they were housed in for experiments to minimize stress from changing environments. Prior to beginning a trial, the plastic bucket containing the cricket being used was transferred into the sound-attenuated room and onto an antivibration table. The cricket was then allowed 10 minutes to acclimate to the room before beginning the trial, then the video camera was switched on. Video footage for each trial was recorded continuously from this point onwards until the end of the trial. Figure 3.3 shows the time sequence of sounds and periods of silence from the beginning to the end of a trial. To establish a baseline for comparison between behaviours before and after a stimulus, I began each trial by video-recording the cricket for at least one minute during a silent

control. To capture freezing responses to predator sounds, the cricket had to be moving when the first sound presentation began. As such, if a cricket did not move within three minutes of beginning the baseline recording, the predator sound was not played, and the trial was discarded. A total of 6 trials from the original 50 recordings were discarded from analysis for this reason. If the cricket was moving after one minute of baseline and before the three minute time allotment had passed, the predator sound was played for the first time and the trial proceeded normally. After the first presentation of the stimulus, I recorded the cricket's behaviour for one minute in silence before presenting the stimulus again. This time, I presented the stimulus regardless of the cricket's starting behaviour. After another minute of recording the cricket's behaviour in silence, I presented the stimulus to the cricket for the third (final) time and ended the trial by recording its behaviour in silence for one additional minute. After the entire trial was completed, I turned off the video camera and measured the cricket's mass. Details on crickets used for experiments can be found in Supplementary Material D. All crickets were only used for a single trial and were returned to the colony afterwards. As all crickets used in experiments were isolated before reaching adulthood and rearing bins are age-separated, there was no chance of re-using a cricket.

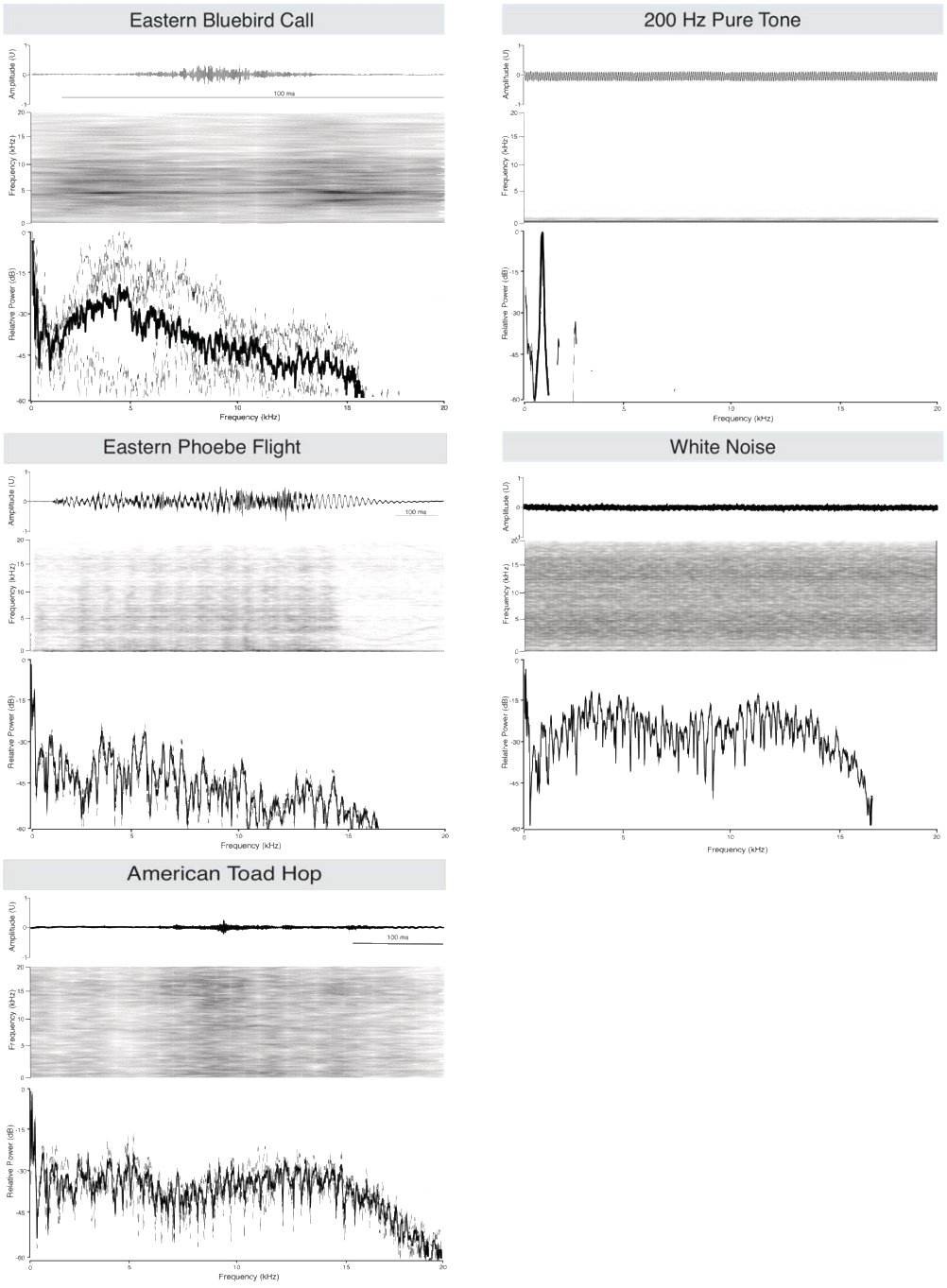

Figure 3.2. Waveforms, spectrograms, and power spectra of sounds used for playback experiments. All sounds were re-recorded in the playback experiment set-up with a dB SPL of 80 dB from the position of the cricket. Bluebird call recordings and toad hop recordings show sounds for recordings from 3 different individuals. Recordings of phoebe flight, the 200 Hz pure tone, and white noise show 3 repetitions of the same sound. Power spectra and spectrograms were generated in R using an FFT of 4096 (Hann window, 50% overlap).

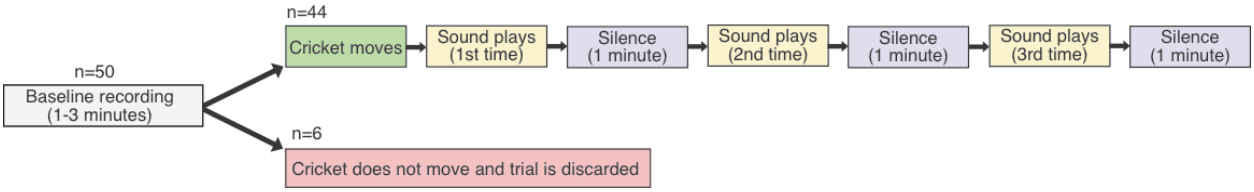

Figure 3.3. Diagram showing the sequence of events for a single playback trial. The top sequence shows a typical trial where the cricket begins moving within 3 minutes of beginning the baseline recordings. If the cricket did not move, the trial was discarded and not used for analysis. The entire sequence was videotaped.

Data Analysis

In this chapter of my thesis, my goal was to assess if crickets use sounds produced by predators for risk assessment. To achieve this goal, I asked the following questions: 1) Do crickets respond behaviourally when presented with sounds?; 2) Does this response differ depending on stimulus type?; 3) Do behavioural responses of crickets change upon subsequent presentations of the sound (i.e. is there evidence for habituation or sensitization)? To answer these questions, I used scored video footage of trials in BORIS (version 7.9, Friard and Gamba 2016) for statistical analysis. Below, I detail how I scored video footage, how raw data from BORIS was organized for figure production and statistical analysis, and the statistical analyses performed for this chapter.

Behavioural scoring

Videos were scored to obtain data to assess responses of fall field crickets to sounds, assess responses to different sounds, and to determine the effects of repeated exposure to predator sounds (i.e., if there is evidence for habituation or sensitization to

sounds). Videos recorded during playback experiments were exported from the Sony video camera as MTS files and uploaded to a laptop computer (2020 M1 MacBook Air, Apple Inc., Cupertino, CA, USA). Video files were then scored in the behavioural observation program BORIS (version 7.9, Friard and Gamba 2016) using the same MacBook Air they were uploaded onto.

Behaviours were all scored as states and could not occur simultaneously. I included the following in the ethogram used for scoring: 1) Walking (i.e., moving at a slow pace); 2) Fleeing (escape behaviour; sudden quick movement). Fleeing was easily distinguished from walking when it occurred as crickets would very suddenly begin moving much faster than if they were walking; 3) Grooming (i.e., crickets using legs to remove dirt and debris from body parts); 4) Freezing (i.e., sudden cessation of movement); and 5) Climbing the walls of the experimental arena. Of these behaviours, fleeing and freezing were considered to be antipredator responses as crickets have been demonstrated to exhibit both freezing and flight responses to predator cues such as vibrations and thumping noises suggested to simulate sounds and vibrations (Table 2.1) (Pollack 2007, Hedrick 2013, Fukutomi and Ogawa 2017, Careau *et al.* 2019, Lu *et al.* 2023). To ensure that scoring was not biased by being able to hear when the stimulus was playing, I initially scored the videos with audio set to mute. Subsequently, I re-scored the video with sound turned on to record the onset and offset of sound playbacks. I scored each presentation of the sound as a different state event with a number corresponding to which presentation of the sound was being scored (i.e., in each trial the first presentation was scored as Sound Repetition 1, the second presentation as Sound Repetition 2, and the third as Sound Repetition 3). This allowed

me to score behaviours for each presentation of the stimulus separately to assess any differences in behavioural responses after multiple exposures to the sound.

Data organization and statistical analysis

The primary goal of this chapter is to investigate if fall field crickets are using sounds to detect predators and assess risk. To address this, I asked if crickets exhibited behavioural responses to playbacks of airborne sounds produced by their predators. I predicted that if crickets are using sounds for risk assessment, they will respond to playbacks of predator sounds with freezing or fleeing responses. Of the sounds used for playback experiments, I predicted that crickets would respond behaviourally to bluebird calls and phoebe flight as they are the predator sounds most likely to overlap with sensitivity of the acoustic sensory organs (see Chapter 2). To test this prediction, I first assessed whether each cricket responded behaviourally to each presentation of the sound stimulus. A behavioural response was defined by a change in behavioural state during the time the sound was being played. In the first presentation of the stimulus, this was always a change from moving to freezing. In subsequent presentations, this change in state could include a change from one behavioural state into any other behavioural state (e.g., moving to freezing, grooming to freezing, stopped to moving). Then, for each stimulus presentation I calculated the proportion of individuals that responded behaviourally and tested for significant differences between proportion of individuals responding using a Kruskal Wallis test in R Studio (R Studio Team 2020). Additionally, I asked if cricket behaviours differed overall after being presented with a predator sound. To answer this question, I exported raw data from the scored trials in

BORIS as .csv files for exploratory statistical analysis. Raw data from BORIS was transformed using Excel to combine data from each individual and to show the proportion of time spent in each of the scored behaviours for each trial separated by if it was during baseline recordings, first, second, and third stimulus presentation. I also calculated the proportion of time spent in each behaviour in the one-minute period of silence separating each presentation of the stimulus to compare to baseline. I used R Studio (R Studio Team 2020) to compute means and standard deviations for the proportion of time spent in each behaviour and proportion of crickets showing freezing responses to the played back sounds.

Results

In this chapter, I investigate if fall field crickets (*Gryllus pennsylvanicus*) use sounds generated by predators for risk assessment. To address this question, I played sounds generated by predators (Eastern bluebird calls, Eastern phoebe flight, toad hopping sounds) and non-predators (200 Hz pure tone, white noise) to assess if crickets respond behaviourally. Each cricket was played three repetitions of a single sound stimulus separated by one minute of silence. I then scored video footage of this behaviour to measure the following: 1) The proportion of crickets that changed their behavioural state in response to each played back sound; 2) The proportion of time spent walking, grooming, climbing, or freezing during the sound stimulus presentations and the one minute of silence following each presentation; and 3) If behavioural responses to sound stimuli changed with subsequent presentations of the stimulus (i.e., if there was evidence for habituation or sensitization).

Do crickets respond behaviourally to playbacks of predator sounds?

Behavioural responses to played back sounds were measured as the proportion of individuals that changed their behavioural state when presented with a sound stimulus. These behavioural changes usually happened within <1 second of the sounds beginning and were only scored as a behavioural change if it occurred before the end of the sound stimulus (<2 seconds). The proportion of crickets that changed behavioural states differed significantly between sound types (Kruskal Wallis χ^2= 11.119, p=0.02526). To assess where these differences occurred, I performed a post-hoc Dunn's test. Due to small sample sizes and power issues, there were no significant differences between groups when correcting for multiple comparisons, but there were some non-significant trends showing crickets responded behaviourally more often to Eastern bluebird calls and Eastern phoebe flight when compared to toad hopping and the 200 Hz pure tone. Crickets also froze for a longer time in response to phoebe flight sounds than any other stimuli (Figure 3.4). In general, overall activity levels of crickets during playbacks and the one-minute silent period following playbacks did not change when crickets were presented with bird predator stimuli (Figure 3.5). However, there was a trend showing that overall activity levels marginally increased when presented with toad hopping or either of the two control stimuli (Figure 3.5).

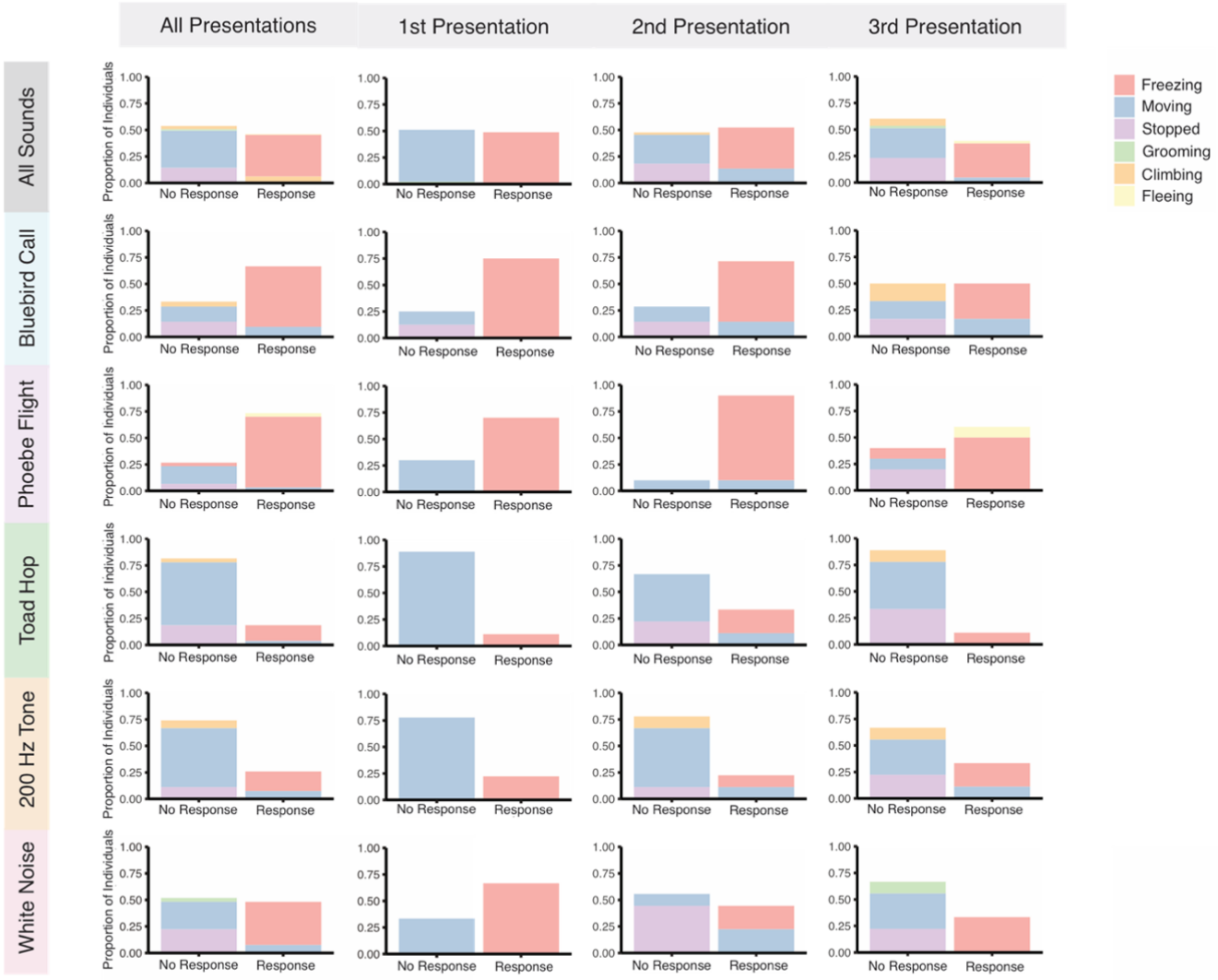

Figure 3.4. Proportion of crickets responding to playbacks of predator and non-predator sound stimuli. Data is shown for the following (left to right): all presentations of each stimulus aggregated together, the first presentation of each stimulus, the second presentation of each stimulus, and the third presentation of each stimulus. The fill of each bar shows the behaviours exhibited by crickets during the stimulus presentation. Sample sizes were as follows: n=44 for all sounds grouped together, n=7 for bluebird call, n=10 for phoebe flight, n=9 for toad hopping, n=9 for 200 Hz pure tone, n=9 for white noise.

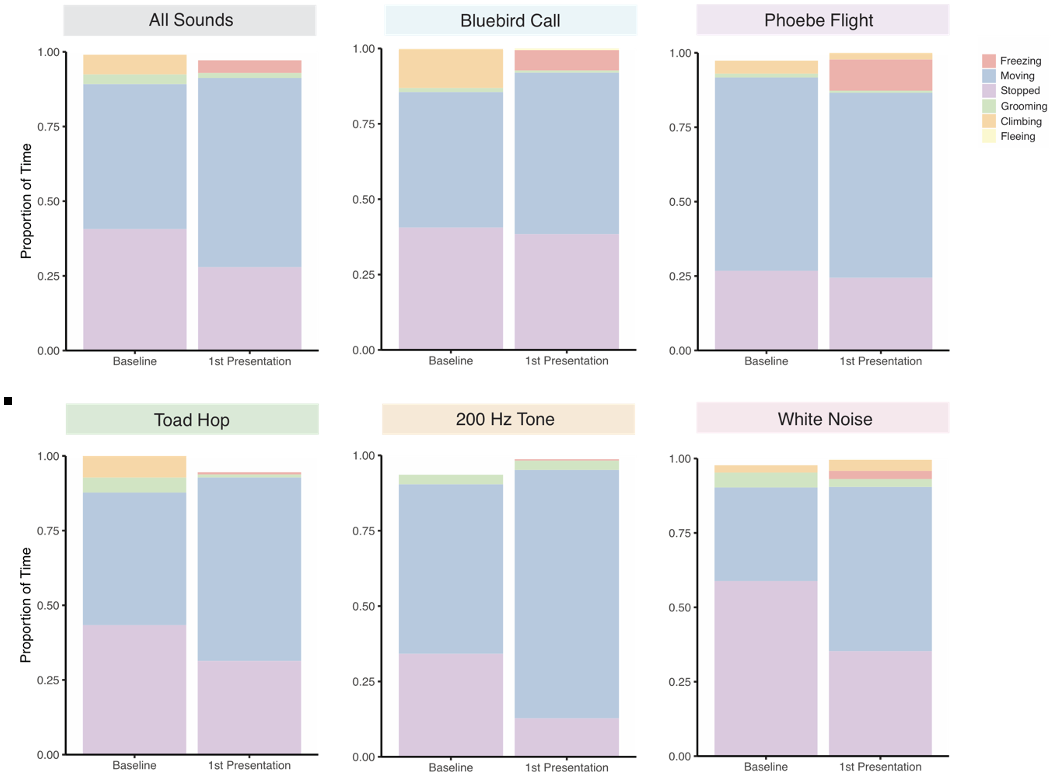

Figure 3.5. Proportion of time spent in each behaviour during baseline recordings compared to during playbacks of predator stimuli. The Period of time behaviours are shown for includes the one-minute silent period following playbacks. Sample sizes are as follows: all played back stimuli (n=44), playbacks of Eastern bluebird calls (n=7), Eastern phoebe flight (n=10), toad hopping (n=9), a 200 Hz pure tone (n=9), and white noise (n=9).

Do behavioural responses vary when presented with a stimulus multiple times?

Overall, there were no significant differences in proportion of individuals responding between subsequent presentations of any sounds, though there was a non-significant trend showing that crickets responded more to white noise when it was presented for a third time (Figure 3.5). This suggests that crickets may become sensitized to some sound stimuli despite there being no significant trends in my thesis.

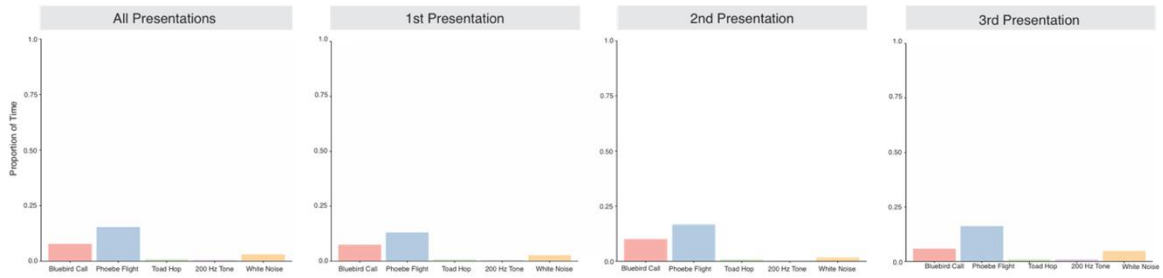

Figure 3.6. Proportion of time spent freezing by crickets (*G. pennsylvanicus*) in three repeated presentations of played back sound stimuli. Mean proportion of time spent freezing is shown for Eastern bluebird call (n=7), Eastern phoebe flight (n=10), American toad hopping (n=9), 200 Hz pure tone (n=9), and white noise (n=9).

Discussion

The primary goal of this chapter was to assess if fall field crickets are responding behaviourally to sounds produced by potential predator species. I predicted that if crickets are using predator sounds for risk assessment, they will respond behaviourally to predator sounds. I tested this prediction in a playback experiment where I measured behavioural responses of crickets that were exposed to three repetitions of a single predator sound. I analyzed video footage of these trials to see if crickets either exhibited antipredator behaviours (e.g., freezing or fleeing) or changed their behavioural state in response to predator sounds. Overall, I found that crickets were more likely to change behavioural state in response to playbacks of sounds produced by avian predator species (i.e., Eastern bluebird calls and Eastern phoebe flight sounds), but not in response to playbacks of sounds produced by foraging toads. Moreover, crickets froze for longer durations in response to playbacks of avian predator sounds than any other tested sound stimulus. Below, I discuss the significance of these predator cues, behavioural responses of crickets to predator sounds cues, and summarize by providing recommendations for future studies.

Behavioural responses to sounds

Freezing is a widespread antipredator response that has been shown to function in risk assessment in many animals, including field crickets (Niemalä *et al.* 2012). Freezing responses are generally characterized by the cessation of all movements except those associated with respiration and vision. By becoming immobile, prey are less likely to be detected by predators, particularly predators that detect prey visually or

by substrate vibrations (Misslin 2003). Freezing generally is the first behavioural response upon detection of a predator that is still far away and has not yet detected the prey (Blanchard and Blanchard 1969). As such, freezing is generally exhibited when risk of predation is still low or moderate and is thought to be a strategy to avoid being detected (Misslin 2003, Adamo *et al.* 2013). In the genus *Gryllus,* freezing has largely been documented in response to vibrations thought to be representative of approaching predators (Adamo *et al.* 2013, Hedrick 2013, Careau *et al.* 2019). Freezing has also been reported in *Gryllus texensis* in the presence of a live predator (Adamo *et al.* 2013). My results show that playbacks of predator sounds induce freezing behaviour in *Gryllus pennsylvanicus*. In my experiment, the first presentation of all stimuli produced a freezing response in at least one individual. However, sounds produced by avian predators initiated a freezing response in more individuals than playbacks of toad hopping or the control non-predator sounds. Considering that all the sounds used in playback experiments were shown to overlap at least partially with field cricket hearing sensitivity in Chapter 2, this suggests that crickets can discriminate between predator sounds and likely are attending more closely to sounds produced by avian predators.

While researchers have documented escape or flight behaviour (i.e., fleeing or jumping away from a perceived predator) in some species of field cricket, these have exclusively been documented either in response to detection of a bat predator by eavesdropping on bat echolocation signals or to wind currents on the cerci indicating an imminent attack (e.g., Pollack 2007, Fukutomi and Ogawa 2017, Lu *et al.* 2023). My results only showed a single cricket eliciting escape behaviour in response to phoebe flight sounds. As escape behaviour is very energetically costly, animals tend to only

exhibit escape behaviour when threat of predation is very high, and an attack is imminent (Pollack 2007). My results showed only a single instance of escape behaviour, suggesting that the acoustic predator cues used in my playback experiments are not strong enough indicators of imminent attack to initiate a flight response in fall field crickets. It is possible, however, that these cues may be used to initiate a flight response in combination with other predator cues. For example, some studies have shown that acoustic predator cues are used to mediate wind-elicited escape in field crickets, meaning that whether crickets initiate a flight response when a change in wind patterns is detected by the cerci is dependent on the acoustic environment (Fukutomi and Ogawa 2017, Lu *et al.* 2023). It is possible, then, that these sounds may play a role in mediating wind-elicited escape responses despite not eliciting flight on their own.

Other behavioural responses to predator sounds

Most of the research on antipredator behaviour in *Gryllus* species has been focused on freezing, fleeing, and hiding behaviour (e.g., Hedrick and Kortet 2006, Niemelä *et al.* 2012, Adamo *et al.* 2013, Hedrick 2013), however, crickets may change other behaviours upon detection of a predator as well. For example, changes in overall amount of time spent moving have been reported to occur when some species of cricket are exposed to predator cues (*Gryllus pennsylvanicus*: Storm and Lima 2008, Storm and Lima 2010; *Nemobius sylvestris:* Binz *et al.* 2014). While in wood crickets (*Nemobius sylvestris*) it has been shown that individuals will either increase or decrease movement in response to predator cues, my study species (*Gryllus pennsylvanicus*) has been documented to decrease movement and speed in the presence of chemical cues

of a spider predator (Storm and Lima 2008, Storm and Lima 2010). While my results generally did not appear to have any significant trends in movement in response to predator sounds, it is possible that there was individual variation in changes in movement not captured in the figures used in this chapter.

Summary and future directions

This chapter presents preliminary evidence that fall field crickets are using sounds produced by potential predators to assess risk and avoid predators. In general, crickets froze in response to playbacks of predator sounds, and more crickets froze in response to sounds produced by insectivorous birds than foraging toads. While non-predator sounds (white noise and a 200 Hz pure tone) also evoked a freezing response in at least one individual, the number of individuals freezing was lower compared to playbacks of bird sounds.

Evidence presented in my thesis showing that fall field crickets may use sounds to detect and avoid predators is limited and more experiments are needed. Firstly, predator sounds should be recorded in a natural environment as well as a lab environment to inform on natural dB levels and bandwidths. Additionally, future studies should include a wider array of predator sounds with a larger sample size. Sounds that may be relevant and useful for playback experiments include rustling leaves, breaking branches, and recordings of predators moving on a larger variety of substrates that crickets may be found in their natural environment (e.g., leaf litter, gravel, grassy substrates). Additionally, it would be useful to play predator sounds to crickets while

recording the tympanal nerve to determine if they respond neurally to predator sounds as well as behaviourally.

This chapter focused on responses of crickets to played back sounds. However, how crickets use solid-borne vibrations to detect predators is also understudied. Time and equipment constraints did not allow for vibrational playbacks to be included in my thesis, but this would be important to test in future studies. While there is some evidence showing that some species of field cricket respond to played back vibrations by freezing (e.g., Dangles *et al.* 2008, Adamo *et al.* 2013, Hedrick 2013, Careau *et al.* 2019), all of these studies used vibrations generated by humans in the lab and did not compare the vibrations used to those actually produced by predators of field crickets. I would recommend that future studies incorporate playbacks of vibrations generated by predators of *Gryllus pennsylvanicus* and include both behavioural and neural measurements to determine if fall field crickets are also using vibrations to detect and avoid predators.

Chapter 4: General discussion and conclusions

There are many examples of insect prey using tympanal hearing and vibratory sense organs to detect and avoid predators. Much of this research, however, has focussed on the detection of echolocation calls of bat predators by flying insects (e.g., Hoy 1992, Miller and Surlykke 2001, Yager 2012, Pollack 2015, 2016) and the detection of oncoming predators through plant-borne vibrations (e.g., Castellano and Barbosa 2006, Gish *et al.* 2011, Guedes *et al.* 2012, Gish *et al.* 2021) despite predators conceivably producing an array of other sounds and vibrations insects may be able to use for predator detection. Such sounds include incidental cues from foraging or movement (e.g., rustling leaves and grass, snapping twigs, flight sounds from aerial predators, escape sounds from other prey) and communication signals that insect prey may eavesdrop on (e.g., predator communication signals, prey alarm signals).

It is reasonable to think that insects may use sounds produced by non-bat predators for risk assessment for a number of reasons. Firstly, there are insects that have ears or hearing sensitivity that with no known purpose. This includes ears present in non-calling insects such as butterflies (e.g., Lane *et al.* 2008, Lucas *et al.* 2009, Sun *et al.* 2019) and some grasshoppers (e.g., Lehmann *et al.* 2010, 2012), praying mantids with hearing tuned to low frequencies (e.g., Yager 1996), and calling insects with hearing sensitivity that does not match their calling songs (e.g., Mason 1991, Yack *et al.* 2000). In these cases, hearing must have another function outside of conspecific communication or detection of bat predators, and it has been suggested that the function of their ears may be to detect non-bat predators (Yack *et al.* 2020). Presently, evidence for insect prey using sounds and vibrations generated by non-bat predators is

limited to the use of incidental flight sounds from parasitoid wasps (e.g., Tautz and Markl 1978, White *et al.* 1983, Gnatzy and Heulein 1986, Rothschild and Bergström 1997, Taylor and Yack 2019), flight and foraging sounds from bird predators in some species of moth (Jacobs *et al.* 2008, Fournier *et al.* 2013), and potentially bird communication signals in some species of *Erebia* butterfly (Ribaric and Gogola 1996). This study provides more evidence that insectivorous predators produce sounds and vibrations that may be useful for predator detection in insects, and specifically, that hearing in a locally abundant field cricket (*Gryllus pennsylvanicus*) may also function to detect and avoid predators.

In field crickets (*Gryllus* spp.), tympanal hearing is vital for detecting conspecifics, either in the context of reproduction or territorial/aggressive interactions (Alexander 1961, Schöneich 2020). As crickets have such well-developed hearing, it is likely that they would function in other contexts, including predator detection, though studies on this have been limited to detecting and avoiding bat predators. For example, some night-flying species of field cricket are known to respond neuronally and behaviourally to playbacks of bat echolocation signals (e.g., Paton *et al.* 1977, Schildberger and Hörner 1988, Pollack and Martins 2007, Schöneich *et al.* 2015, Hedwig 2016). However, crickets are prey to a variety of non-bat predators, and non-flying species of cricket may be more likely to use cues from other predators to avoid capture. My thesis provides evidence that a local species of field cricket (*Gryllus pennsylvanicus*) uses sounds produced by avian predators for predator detection and risk assessment. Communication calls produced by the Eastern bluebird (*Sialis sialis*), a local insectivorous bird known to eat crickets, overlap with hearing sensitivity of *Gryllus*

pennsylvanicus. Additionally, flight sounds from another local insectivorous bird species, the Eastern phoebe (*Sayornis phoebe*) overlapped with sensitivity of low-frequency detecting units in the cricket acoustic sensory complex, meaning *G. pennsylvanicus* can also detect sounds produced by bird predators approaching in flight. In playback experiments using bluebird calls and phoebe flight sounds, crickets responded behaviourally to these sounds by freezing. This suggests that *G. pennsylvanicus* uses sounds produced by avian predators to avoid predation. While crickets did not respond behaviourally to sounds produced by hopping toad predators, it is possible that they may use related substrate vibrations from oncoming toads for risk assessment. Vibratory playback experiments, however, were not included in my thesis.

While not directly related to my thesis, I also recorded airborne and vibrational acoustic signals produced by *Anaxyrus americanus* during handling, termed release calls and release vibrations respectively. Many species of toad, including American toads, have been documented to produce release calls and vibrations when unreceptive to a mating attempt (Schmidt 1972, Sullivan 1992, Leary 1999). Interestingly, release calls have also been documented in predator-prey interactions when toads are captured by snake predators (Marchison and Anderson 1978). This suggests that these signals may function in antipredator contexts as well as reproduction. While release calls have been previously characterized (Sullivan 1992), release vibrations have not. My thesis provides the first characterization of release vibrations of the American toad.

While my thesis provides evidence for acoustically mediated risk assessment in the fall field cricket, I recommend some experiments to build upon the results presented here. First, there is a wide array of other predator cues that an insect may use for risk

assessment aside from those characterized in Chapter 2. For example, insects may eavesdrop on alarm signals or escape sounds from non-predators, or a variety of other predator communication signals. Additionally, my field recordings were limited due to equipment issues and high levels of anthropogenic noise. I recommend that recordings of additional predator signals and cues be performed in an insect's natural environment to determine if they are available to insect prey under natural conditions. Second, my playback results are limited due to small sample sizes and equipment constraints that prevented vibration playbacks. I recommend that more playback experiments are conducted to allow more sophisticated analysis of behavioural responses of insects to predator cues, as well as playbacks using vibrational cues. Overall, it is very likely that sounds and vibrations produced by non-bat predators provide important information for predator detection and risk assessment in insects. While this question has been receiving more attention recently, very little is still known about how insects may use such acoustic cues to avoid predation, and more research is needed to understand how important these cues may be to an insect's survival.

Supplementary Materials

Supplementary Material A. Sex, mass, and location of capture for live toads (*Anaxyrus americanus*) recorded in Chapter 2.

Toad ID #	Date captured	Coordinates of capture location	Sex	Mass (g)	Recording location
1	August 16, 2022	45.385170, -75.685954	Female	NA	Lab
2	August 16, 2022	45.385170, -75.685954	Male	NA	Lab
3	August 17, 2022	45.385170, -75.685954	Female	NA	Lab
4	August 22, 2022	45.385170, -75.685954	Female	56.1	Lab
5	August 22, 2022	45.385170, -75.685954	Male	21.5	Lab
6	August 24, 2022	45.385170, -75.685954	Female	43.0	Lab
7	August 24, 2022	45.385170, -75.685954	Male	30.5	Lab
8	August 25, 2022	45.385170, -75.685954	Male	33.6	Lab
9	August 26, 2022	45.385170, -75.685954	Male	30.4	Lab
10	August 29, 2022	45.350656, -75.816121	Male	39.5	Lab
11	August 29, 2022	45.350656, -75.816121	Male	39.9	Lab
12	August 29, 2022	45.350656, -75.816121	Male	30.8	Lab
13	September 19, 2022	45.350656, -75.816121	Male	34.1	Field
14	September 19, 2022	45.350656, -75.816121	Female	41.2	Field
15	October 12, 2022	45.350656, -75.816121	Male	19.8	Field

Supplementary Material B. Live predators and sound playbacks of predators characterized in Experiment 1 and presented to live crickets in Experiment 2.

Stimulus	Samples		Source of recording	Comments
	Lab	Field*		
American toad movement	13	3	Recorded live in-lab and in the field	Sounds and vibrations recorded separately. Recorded with crickets present, sometimes while capturing crickets.
Bluebird song (playback)	3	NA	Sourced online from xeno canto	Conspecific communication signal used to attract mates.
Bluebird call (playback)	3	NA	Sourced online from xeno canto	Call often made when bringing food to nestlings
Phoebe flight sounds (playback)	1	NA	Recorded by former Yack lab members	Flight sounds produced in pursuit of flying insect prey.
Breaking branches (playback)	1	NA	Recorded by former Yack lab members	Sounds produced by breaking of branches by hand.
Masses dropping (live)	5	NA	Recorded live in lab	Two masses used here. Mass 1 is 1.02g and Mass 2 is 95.4g. Sounds and vibrations recorded to be used as a baseline.

Supplementary Material C. Sound level measurements for stimuli played back in Chapter 3. The volume of each played back sample was calibrated prior to being played back to crickets so the sound was within the range of sound pressure levels reported in this table. When a range is given, it represents the lowest and highest sound level measurement taken for the sample used for playbacks.

Stimulus	Sound level range (dB SPL)
Eastern bluebird (Sialis sialis) call	65-80
Eastern phoebe (Sayornis phoebe) flight	70-80
American toad (Anaxyrus americanus) hopping	60-70
200 Hz pure tone	70
White noise	70

Supplementary Material D. Sex and mass for each cricket used in playback experiments in Chapter 3. Note that this table does not include individuals whose trials were discarded due to inactivity, but does include individuals whose trials may have been discarded for other reasons such as background noise

Cricket ID	Sex	Mass (mg)
A1	Male	170.4
A2	Female	349.6
A3	Female	312.5
A4	Female	238.9
A5	Male	272.7
A6	Female	389.6
A7	Male	256.3
A8	Male	258.4
A9	Female	347.2
A10	Female	312.5
A11	Female	319.3
B1	Male	237.8
B2	Female	423
B3	Female	419.4
B4	Male	222.4
B5	Male	224.4
B6	Male	207.6
B7	Female	414.6
B8	Male	138
B9	Female	388.9
B10	Female	355.6
C1	Male	183.9
C2	Male	229.7
C3	Female	303.3
C4	Female	280.5
C5	Female	376.9
C6	Male	226.5
C7	Male	214.4
C8	Male	350.4
C9	Female	235.5
C10	Male	176.2
D1	Male	211
D2	Male	235.4
D3	Female	347.1
D4	Male	159.6
D5	Female	338.9
D6	Male	246.6
D7	Female	652

D8	Female	546.6
D9	Male	271.8
D10	Female	318.5
F1	Female	576.1
F2	Female	398
F3	Male	272.5
F4	Female	528.3
F5	Female	372.2
F6	Female	428.4
F7	Male	260.9
F8	Female	537.6
F9	Female	348.1
F10	Male	250.9

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