SOUND PERCEPTION IN FEMALES OF A FIELD CRICKET, *Acanthogryllus asiaticus*

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A dissertation submitted for the partial fulfilment of BS-MS Dual Degree in Science

Under the guidance of

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26th April 2019

Certificate of Examination

This is to certify that the dissertation titled "Sound perception in females of a field cricket, *Acanthogryllus asiaticus*" submitted by Mr. Jain P K (MS14130) for the partial fulfilment of BS-MS dual degree programme of the institute, has been examined by the thesis committee duly appointed by the Institute. The committee finds the work done by the candidate satisfactory and recommends that the report is accepted.

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Dated: April 26, 2019

Declaration

The work presented in this dissertation has been carried out by me under the guidance of Dr Manjari Jain at the Indian Institute of Science Education and Research, Mohali. This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any other university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgements of collaborative research and discussion. This thesis is a bonafide record of original work done by me and sources listed within have been detailed in the bibliography.

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In my capacity as the supervisor of candidates project work, I certify that the above statements by the candidate are true to the best of my knowledge.

Dr. Manjari Jain

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Dated: April 26, 2019

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LIST OF TABLES AND FIGURES

Fig 1.1 : Cricket call visualisation.

Fig 1.2: Cricket ear.

Fig 1.3: Male and female *A. asiaticus* with its biological classification

Fig 2.1: Playback setup

Fig 2.2: Arena for phonotaxis experiment.

Fig 2.3.1: Responses of female towards different SPLs under ambient noise condition

Fig 2.3.2: Responses of female towards different SPLs under traffic noise condition

Fig 3.1: Representation of a two-choice arena

Fig 3.2: The responses towards differentially loud stimuli.

Fig 4.1: Three sites where the transmission experiments were conducted.

Fig 4.2: Measuring SPL during the experiment

Fig 4.4: Transmission of cricket sound along with different noise conditions.

Fig 4.5: Spectral representation of *A. asiaticus* LDMC with average acoustic parameters under different noise conditions.

LIST OF TABLES

Table 4.3: Habitat wise transmission ranges

TABLE OF CONTENTS

ABSTRACT..1

1. General introduction on cricket intersexual signalling behaviour 2

Communicating for finding a partner 2

Model organism 4

Crickets 4

Cricket calls 4

Call parameters and its characteristics 4

Hearing 6

Study species 6

Bibliography 8

2. How loud must the male be to be detected? Determining the behavioural hearing thresholds of females in quiet and noisy conditions..11

Objectives 13

Methodology 13

Rearing and maintenance of cricket culture 13

.... 13

Playback set-up and settings 13

Arena 14

Phonotaxis trials (No choice) 15

Pilot experiments 16

Experimental protocol 16

Statistical analysis 17

Results 18

Behavioural hearing threshold under normal noise conditions 18

Behavioural hearing threshold under traffic noise condition 18

Discussion 19

Bibliography 20

3. How much louder must the male be to be chosen? Female mate-choice in relation to call amplitude 23

Objectives 24

Methodology 24

Rearing and maintenance of culture 24
Playback set-up and settings 24
Phonotaxis trials (two-choice paradigm) 25
Arena 25
Experimental protocol 25
Statistical analysis 26
Results 26
Discussion 27
Bibliography 29
4. How far can the male be heard? Sound transmission in different environmental conditions 31
Objectives 34
Methodology 34
Study sites 34
Experimental protocol 35
Statistical analysis 35
Results 36
Discussion 37
Bibliography 38
5. Conclusions and future directions 41
Bibliography 42

ABSTRACT

Animal communication is the process in which a sender sends a signal with some information encoded in it in order to evoke a response in the receiver. Communication can take place by different sensory modalities including sound. Crickets are nocturnal insects known for their loud and conspicuous calls. In crickets, stationary males send acoustic signal to silent females who locate them and choose one of many signallers. They exercise their choice by moving towards the chosen male guided by sound alone. This response in the form of movement towards the sound is called phonotaxis. During phonotaxis, females may consider various temporal and/or spectral parameters of calls and its loudness for choosing their potential mate. However, in order to approach the males, females must first detect, recognise and finally respond to the calling males. This may be rendered futile if the female fails either of the preceding steps. One important factor that determines the detection of a call is its loudness, while recognition typically involves a combination of temporal and spectral features of the call. The minimum sound pressure level at which the receiver behaviourally responds to the calls of a sender is called the behavioural hearing threshold.

In this study, I have examined the Behavioural Hearing threshold of the females of Acanthogryllus asiaticus, a field cricket, in ambient and traffic noise conditions to examine whether and by how much traffic noise could alter detection thresholds of mating signals in the insect.. I also examined the transmission of the cricket call in different habitats and then estimated the effective broadcast area by measuring the distance at which the signal strength falls at the ambient noise level. Finally, I examined female mate preference based on loudness in order to see whether females prefer louder males within their limited audible range. The findings suggest that the BHT shifts drastically higher in noisy traffic conditions. The study also predicts a drastic reduction in transmission range in traffic noise conditions as compared to ambient night time noise. It is thus expected that males calling from loud noisy condition must, on an average, have louder calls than those that call from quiet habitats to be even heard by females. These results emphasize the strong negative impact of anthropogenic noise on cricket hearing and signal transmission. I also find that within detection thresholds, females prefer louder males. This was true for males that were 6 dB louder than the males calling at threshold limits but not for males that were only 3 dB louder. The study has important implications on the signalling system in this field cricket species from both the sender and receiver perspective.

1. General introduction on cricket intersexual signalling behaviour

Communicating for finding a partner

Acoustic communication in the animal kingdom can occur in the context of an array of behavioural functions such as mate choice, kin recognition, territory defence and parent-offspring communication signals (Kroodsma & Miller 1982; Searcy & Andersson 1986; Catchpole & Slater 1995; Boncoraglio & Saino 2007). The ability to carry information over longer distances makes acoustic communication suitable for attracting potential mates which are not in view (Bradbury & Vehrencamp. 1998). Nocturnally active organisms such as crickets are one of the prime exemplars of this function of sound (Huber *et al.* 1989). Even though there is an intrasexual selection for mating (through male-male competition such as fighting), intersexual selection relies on the ability of the male to attract and advertise its quality since females are the choosier sex in many animals including crickets (Trivers, 1972; Huber *et al.* 1989). It is also possible that the male signal is optimised depending on the female choices (Huber *et al.* 1989). This shows the importance of examining female preferences for male mating signals in order to understand the quality of these signals.

The studies conducted on crickets suggest that females prefer certain acoustic parameters such as temporal structure (time dependent acoustic parameters such as syllable repetition rate), spectral features (carrier frequency) and loudness inmaking mate choice decisions (Popov *et al.* 1974, Thorson *et at.* 1982, Huber *et al.* 1989). These parameters often have a combined effect on female choice which makes it difficult to study them independently (Huber *et at.* 1989). Regardless of this fact, it was seen in many species such as mole crickets (Forrest. 1983), natterjack toads (Arak 1988), wax moths (Jang and Greenfield 1996) and Jamaican field crickets (Pacheco and Bertram 2014) that females preferred louder male calls. This highlights the importance of signal amplitude in determining female choice, thereby making it relevant to study the perception of loudness of a mate attraction call by female crickets. The loudness and sensitivity towards it, together determine the effective range of transmission of a male's call by elevating the probability of detection of the call by females. This is because on one hand a louder signal will transmit further in a habitat and additionally, if females are sensitive even to weak signals then they will detect further away males. A male cricket which is capable of producing a louder call may benefit in attracting more number of females by increasing the transmission range (Parker 1982; Parker 1983). Apart from this passive attraction, louder males may be preferred over softer ones by females when both males are detectable (Burk 1988; Ryan 1988). The choices are made by the female by looking at the energy expenditure for signal production. Females will prefer males with loud calls, which is possible only for the energetic males (Burk 1988; Ryan 1988). Therefore, a male with relatively higher loudness will gain more number of attracted females by virtue of long range transmission and active female choice.

The first step of sound (signal) perception is the detection of signal followed by recognition and production of suitable responses. The loudness required for signal detection can be termed as the 'hearing threshold'. Hearing threshold itself can be further divided into three; Mechanical Hearing Threshold, Neuronal Hearing Threshold and Behavioural Hearing Threshold (BHT). The detection of the call is only possible if the signal reaches the receiver intact with sufficient information. Therefore hearing thresholds are prone to shifts under altered noise conditions (Blickley and Patricelli 2010).

One of the major factors which can interfere with information transfer through acoustic signals is the influence of ambient noise (Klump, 1996; Slabbekoorn, 2004). This noise can be from anthropogenic sources, heterospecific calls, conspecific calls and the abiotic natural noise which is termed as geophony. Anthropogenic noise poses a major threat to the animal kingdom Yet, its effect on animals is less understood when compared to other threats such as habitat fragmentation and the introduction of invasive species (Blickley and Patricelli 2010). The impact of anthropogenic noise on the behaviour and reproductive success of animals was largely under-reported in the past but has gained a lot of scientific attention in recent times (Kaseloo and Tyson 2004; Dooling and Popper 2007, Blickley and Patricelli 2010). The impact of these noises can be seen in many aspects of animal physiology and behaviour such as trouble in hearing, chronic stress, masking of mating-related signals and hindrance in mating (Blickley and Patricelli 2010). In the same context, the BHT is also prone to shifts which can be either temporary or permanent under the influence of anthropogenic noises such as automobile traffic, construction and other sources (Blickley and Patricelli 2010). This study aims to examine the perception and transmission of acoustic signals, the response from receivers and how the perception and transmission are altered in noisy environments. I used a nocturnal insect, field cricket, as the model organism. Specifically, the objectives of the study were as follows:

1) To examine the Behavioural Hearing Threshold (BHT) of *Acanthogryllus asiaticus* in normal ambient noise conditions and in elevated traffic noise conditions

2) To see whether louder long-distance mating calls (LDMC) are preferred by female *A. asiaticus* and the degree of relative increase necessary for the choice to be exhibited
3) To characterise sound transmission of *A. asiaticus* LDMC in different noise conditions.

Model organism

Crickets

Crickets are nocturnally active insects which predominantly rely on acoustic communication. The calls are produced by males through a mechanism termed as stridulation (rubbing of body parts with each other). The sound production involves sweeping of the hardened edge of the right forewing, called the plectrum over teeth-like structures present on the underside of a vein on the left forewing (Pierce 1948) and amplified by other structures on the forewings known as harp and mirror (Bennet-Clark 1970; Nocke 1971; Michelson and Nocke 1974). The ease of maintaining them in the lab make crickets a good model organism to study under controlled conditions.

Cricket calls

The different signals which are produced by the crickets can be categorised into four;

1. Long Distance Mating Call (LDMC) (Alexander 1962): These are loud calls produced by males to attract conspecific female crickets over several meters.
2. Courtship call (Alexander 1962): The male switches to a softer courtship call when the female appears in the vicinity of the male
3. Postcopulatory calls (Alexander 1962): Calls which are made after a successful copulation
4. Aggressive call (Alexander 1962): produced during aggressive interactions between two males.

Call parameters and its characteristics

A cricket call can have different acoustic parameters such as loudness (sound pressure level or amplitude), temporal (time-dependent parameters such as chirp rate and syllable repetition rate) and spectral (frequency related parameters such as carrier frequency, bandwidth and harmonics) features. The basic unit of one call can be termed as a syllable which combines to form a chirp. Following are the main acoustic parameters of cricket calls (Fig 1.1):

- Temporal parameters
    - Syllable duration: time duration between the onset and offset of a syllable
    - Syllable period: time duration between the onset of one syllable to the onset of the next
    - Number of syllables per chirp
    - Chirp duration: time duration between the onset and offset of a chirp
    - Chirp period: time duration between the onset of one chirp to the onset of the next
    - Chirp repetition rate: the inverse of the chirp period
- Spectral parameters
    - carrier frequency: the frequency at which most of the energy is concentrated
- Amplitude
    - SPL: The sound pressure level gives a measure of the amplitude or energy content of the call produced (measured in dB)

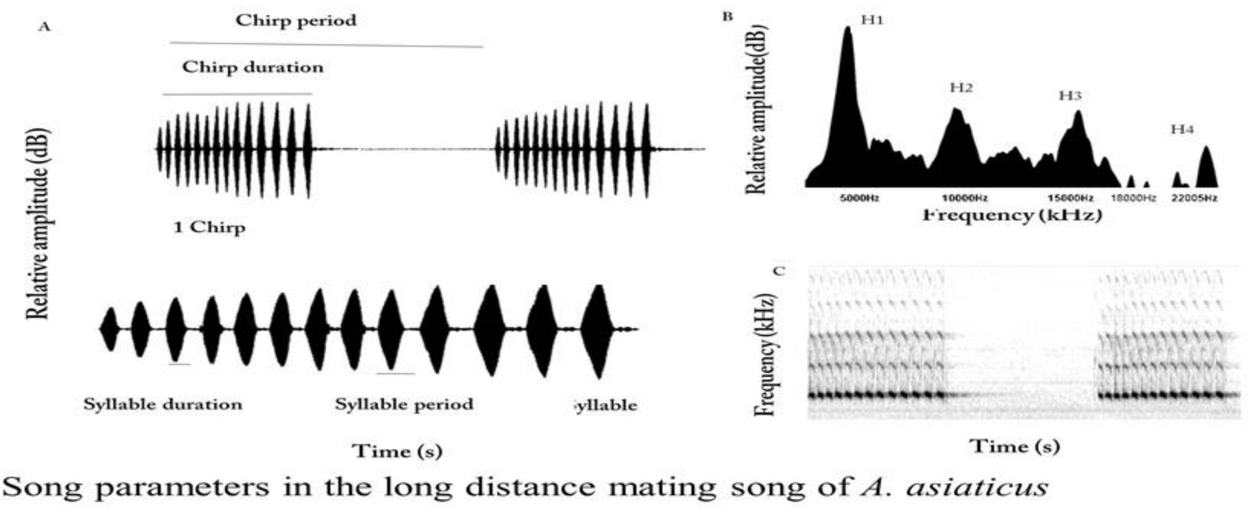


Fig 1.1: Cricket call . A- Oscillogram showing temporal parameters. B- power spectrum showing spectral and loudness parameters. C- spectrogram showing spectral parameters.

Hearing

Cricket ear is considered to be one of the most complex auditory structures ever evolved (Huber *et al.* 1989). The major component of cricket ear is the posterior tympanal membrane which is the pressure receptor (Huber *et al.* 1989). The directionality is attained by the pressure difference (obtained from the sound signal) between ipsilateral (same side of sound source) and contralateral (opposite side of sound source) spiracles and ipsilateral and contralateral tympana (Michelsen *et al.* 1994).

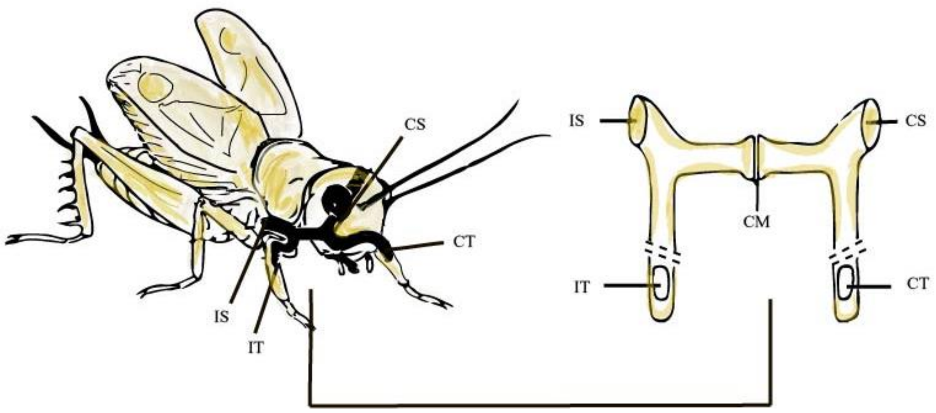
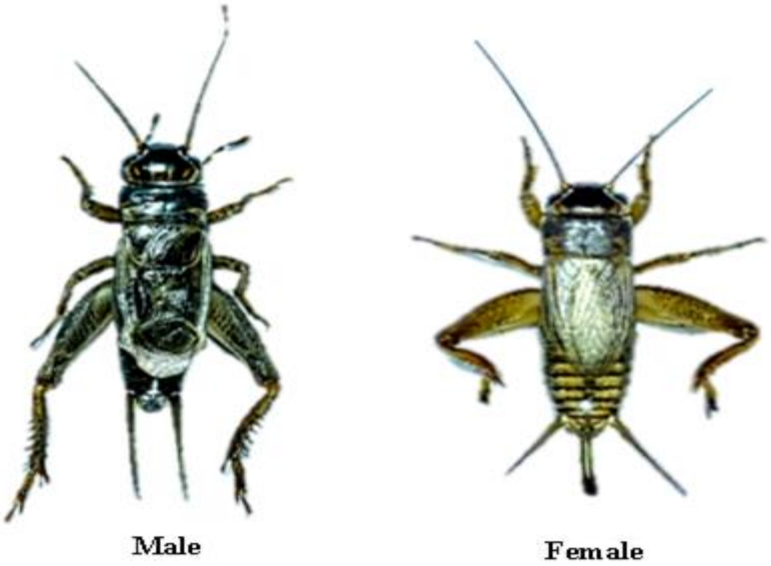


Fig 1.2: Cricket ear. CS- contralateral spiracle, IS- ipsilateral spiracle, CT- contralateral tympanum, IT- ipsilateral tympanum (Adapted from Michelsen *et al*. (1994) and Michelsen and Löhe (1995).)

Study species

I used *Acanthogryllus asiaticus* as the model system as it is a widely available species on IISER Mohali campus and it is easy to handle and breed in lab condition. The species was described by Gorochov in 1990. The classification of the species is as follows.



Kingdom: Animalia

Phylum: Arthropoda

Class: Insects

Order: Orthoptera

Suborder: Ensifera

Superfamily: Grylloidea

Family: Gryllidae

Genus: *Acanthogryllus*

Species: *A. asiaticus*

Fig 1.3: Male and female *A. asiaticus* with its biological classification (picture credit: Nakul Raj)

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2. How loud must the male be to be detected? Determining the behavioural hearing thresholds of females in quiet and noisy conditions

The easily detectable variations in the pressure component (by which the sound is produced) over longer distances makes acoustic communication suitable for long range communication. A sensor which can translate this mechanical source of information into a biologically relevant form will enable the animal to use this method as its method of signal transmission over longer distances (Windmill and Jackson, 2016). The tympanum or tympanal membrane, is a thin membrane of cuticular tissue stretched over a fluid-filled or air-filled cavity in insects to sense these vibrations in the medium (Windmill and Jackson, 2016). This information are then converted into neuronal signal by the associated motion of mechanosensory receptors / neurons which are attached to the tympanum (Windmill and Jackson, 2016). The neuronal inputs are processed subsequently and the animal behaviourally responds or ignores the signal depending upon the information it extracts from the signal (Hoy 1989). One should be aware of the fact that the main function of the insect ear is not to interpret the entire soundscape as it is, but to recognise biologically relevant information for them (Ronacher 2016). Therefore, courtship signals, one of the unavoidable information source for the animals, are also transmitted acoustically among insects (Hoy 1989). If one breaks down this cascade to three processes; detection (sensing the presence of the signal), recognition (discriminating between biologically relevant and irrelevant stimuli) and response (behavioural response to the signal), the completion of the cascade may be rendered futile if the receiver fails either of the preceding steps.

One important factor that determines the detection of a call is its loudness, while recognition typically involves a combination of temporal and spectral features of the call (Huber *et al.* 1989). Each of these steps in the cascade of signal processing requires a threshold of loudness to function which can be termed as the hearing threshold. The sound pressure level (SPL) above which the animal starts detecting a particular sound can be used as a quantitative measure of the hearing threshold. Examining this threshold of loudness for each of these processes will help in understanding the behavioural and physiological aspects of animal. Upon assigning thresholds for each of these processes one can categorise hearing threshold into Mechanical Hearing Threshold (SPL above which the tympanal membrane starts vibrating in response to sound pressure) (Autrum, 1941; Field et al., 1980;

Lomas *et al.* 2011), Neuronal Hearing Threshold (SPL above which the auditory neurons start responding) (Capranica and Moffat 1975; Walkowiak 1980; Ryan *et al.* 2018; Balakrishnan 2006) and Behavioural Hearing Threshold (BHT) (SPL above which the animal starts behaviourally responding towards the call) (Mhatre and Balakrishnan 2006; Ryan *et al.* 2018). The former two might give information to the animal about its surroundings but it need not necessarily be something biologically relevant to it. On the other hand, the latter might give us inferences about the stimulus and its role in communication, as these calls have functional implications in the animal's behaviour such as mate finding and mate choice. Behavioural assays such as phonotaxis (examining the movement of receivers towards a sound source) could be employed to measure the BHT while Laser Doppler Vibrometry and invasive electrophysiological experiments are conducted for testing the mechanical and neurological hearing thresholds respectively (Autrum, 1941, Ryan *et al.* 2018).

In a natural scenario, the acoustic signals will be transmitted through an environment which already has a mix of relevant and irrelevant signals for the receiver. These compositions of sounds can be categorised into three; "biophony"- the sound which is created by other organisms (which includes conspecifics and heterospecifics), "geophony"- the sound from various non-biological sources (such as the whistling of wind, rustling of leaves, flow of water) and "anthrophony"- sounds created by humans (such as drilling, mining or traffic noise) (Bryan *et al*. 2011). All of these sounds could interfere with the signals and hence these are considered as noise for an individual signaller.

Among these noises, anthropogenic noise is something which needs to be addressed seriously because of its expanding influence due to rapid urbanization (Gurule-Small and Tinghitella, 2018). It has been shown that anthropogenic noise affects animals behaviourally as well as physiologically (Barber et al. 2009; Wright et al. 2007; Kight and Swaddle. 2011; Kunc, McLaughlin, Schmidt. 2016; Morley, Jones, Radford. 2014). Studies on invertebrates (Morley *et al.* 2014), anurans (Bee and Swanson 2007), aquatic animals (Kunc *et al.* 2016) and birds (Patricelli and Blickley 2006) show the effects of anthropogenic noise on animal signalling. These impacts can persist throughout the lifetime of an individual animal such as impaired hearing by permanent threshold shift (PTS) or a reduction in hearing sensitivity for a short duration of time which is termed as temporary threshold shifts (TTS) (Rabin and Greene. 2003). It has been seen that very high noise is required to cause PTS and TTS in birds (Blickley and Patricelli 2018). In this study, I specifically examined the behavioural hearing threshold and the impact of anthropogenic noise on BHT of a field cricket; *Acanthogryllus asiaticus*.

Objectives

The major aim of this study was to specifically look at the influence of environmental noise on the BHT of females crickets using *A. asiaticus* as a model system. The specific objectives are given below:

1. To examine the Behavioural Hearing Threshold (BHT) of female *Acanthogryllus asiaticus* in quiet conditions in absence of bio, geo or anthrophony.
2. To examine the BHT of the females in presence of anthrophony (traffic noise).

Methodology

Rearing and maintenance of cricket culture

The crickets used were taken from the lab culture which was maintained under stable environmental conditions. The temperature was maintained at 24°C. The crickets were provided 12 hours light and 12 hours dark conditions to maintain their circadian rhythm. They were provided food and water *ad libitum*. Empty egg cartons were placed in plastic containers to provide shelter. After the final moulting, individuals were segregated and kept in separate plastic containers with food and water. The date of final moulting along with the individual ID was recorded on each container. The food and water, as well as the containers, were cleaned at regular intervals.

Playback set-up and settings

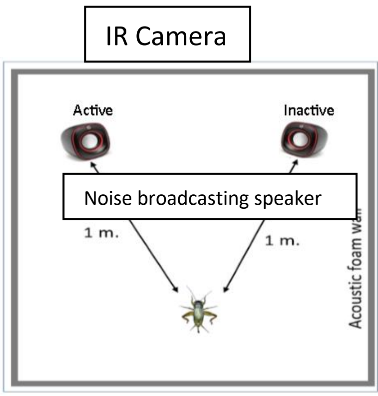

Fig 2.1: Playback set-up.

The playback experiments were conducted in a phonotaxis arena in a single choice paradigm. The call stimulus (having temporal and spectral parameters reflecting the population average) was taken from a library of cricket calls recorded from the field (Singh and Jain unpublished). Calls were played from either one of two JBL GO speakers, placed 1m away from the point of release at an angle. These speakers were separated from each other by a distance of 1m. The SPL of the calls were measured using a Bruel & Kjaer ½'' microphone, Type 4189 (20 Hz to 20 kHz) which is attached to a Sound Pressure Level Meter, Type 2730 (Bruel & Kjaer, Naerum, Denmark) at a distance of 0.5 m from the active speaker. The test stimuli differed in SPL, which were 22dB, 30dB, 38dB, 42dB, 46dB and 56dB and were played back, starting from the softest to the loudest from one of the speakers placed in the arena. This was done because of the possible interference of louder calls in animal's behaviour if those calls were played at the beginning. The left and right speakers were switched in alternate trials for the same individual to control for side bias. Each playback trial lasted for 5 minutes.

Arena

An arena of 1.8 m * 1.8 m * 1 m was built with acoustic foam, 7cm in thickness, placed as its walls, in order to reduce the ambient noise condition. The floor of the arena was covered with soil. Crickets were released using the help of a pulley system and a plastic container which was placed at the centre of one edge of the arena. The videos of the trials were recorded from a fixed position from outside the arena.

Figure 2.2: Arena for phonotaxis experiment. Two speakers (right) and female releasing point (left) can be seen in the photograph.

Phonotaxis trials (No choice)

The movement of an organism in the direction of a sound signal is called phonotaxis. The phenomenon allows one to evaluate signal preference by examining various features of the phonotaxis, including approach latency (the time taken to initiate approach towards the sound source), phonotaxis duration (time elapsed from the beginning to the end of the phonotaxis) and the path taken for approach. The BHT of the crickets were tested using phonotaxis experiments following a ‘No-choice paradigm’, where they were exposed to only one signal at a time to which they could either respond or not. The trials were conducted in a phonotaxis arena. A second identical speaker was used as a control for visual cues. The females were released 1 m away from the speaker using a pulley system after 1 minute of acclimatisation under a plastic container to the experimental conditions present in the arena. Each of the individuals was exposed to the calls for 5 minutes. They were considered to be responding to the stimulus if they reached at least 15 cm near to the active speaker within the given time. Otherwise, it was recorded as a no response.

The protocol for phonotaxis experiments examining the BHT under normal conditions were also followed for examining BHT under noisy conditions.

Pilot experiments

53 pilot trials were done to standardise the experimental setup. Different stimuli were played to each animal in a random order. The speakers were placed in the corners of the arena and the female cricket was released 1.5 m away from the speakers such that both speakers were facing the female front forward and were equidistant to the release point. It was found that exposing females to the louder calls (stronger stimulus) at the beginning alters their behaviour during the following trials. We reorganised the order of exposure of calls from softest to loudest (from 22 dB to 54 dB) later during the actual trials. We also placed the speakers 1m away from the point of release of crickets to mimics sound-at-source conditions to rule out confounding effects of excess attenuation (attenuation of sound in excess to 6 dB loss due to spherical spreading.

Experimental protocol

Socially and acoustically isolated adult *A. asiaticus* were used in the trials. These crickets were tested in phonotaxis experiments in single choice paradigm. A total of 20 individuals were exposed to various stimuli with sound pressure levels varying from 22 dB to 54 dB in the order of increasing loudness. Experiments were carried out from 7 pm to 11 pm (peak activity time of the crickets). One female was tested only twice per night and given at least 24 hours gap before subjecting it to next set of trials. After every trial, the side of stimuli exposure was swapped to control for the side bias and the soil was shuffled to remove the olfactory cues. If the female was not responding towards one stimulus, we checked for the motivation level of female (positive control) by exposing it to a call of an average SPL (62dB). A negative control experiment was performed with an active speaker with a silent stimulus and used this to compare the results. Similar experimental protocol was followed for finding BHT under traffic noise except that an additional speaker was fixed 1 m above the centre of the arena to play back traffic noise. The noise across the floor of the arena was not significantly different and it was lower than the room's average ambient noise level (the noise level ranged from 9 dB to 11 dB (±0.89) inside the arena). The response of the individual, latency of the response, loudness of the stimuli and the side of exposure was recorded in a datasheet.

Statistical analysis

All the statistical analyses were done using Statistica (64 bit, version 12.7.207.0). The female's behaviour towards an experimental stimulus was recorded as a response or no response. This data was statistically tested using χ^2 p-value test. The responses towards each SPL were compared with the silent control. The least loudness in which response was significantly higher than the silent control was considered as the behavioural hearing threshold.

Results

Behavioural hearing threshold under normal noise conditions

Out of seven experimental treatments 46 dB, 54 dB and 62 dB showed significantly higher positive responses **(**Chi-square (df=1)=10.99, P=0.0009, Chi-square (df=1)=12.91, P=0.0003 and Chi-square (df=1)=10.99, P=0.0009 respectively**)** than the silent control (N = 20 individuals for each experimental and control stimuli). 22 dB, 30 dB, 38 dB and 42 dB did not show significant difference from the silent control **(**Chi-square (df=1)=0, p=1.0000; Chi-square (df=1)=0.36, P= 0.5483; Chi-square (df=1)=0, P=1.0000; Chi-square (df=1)=3.58,P= 0.0583 respectively**)**

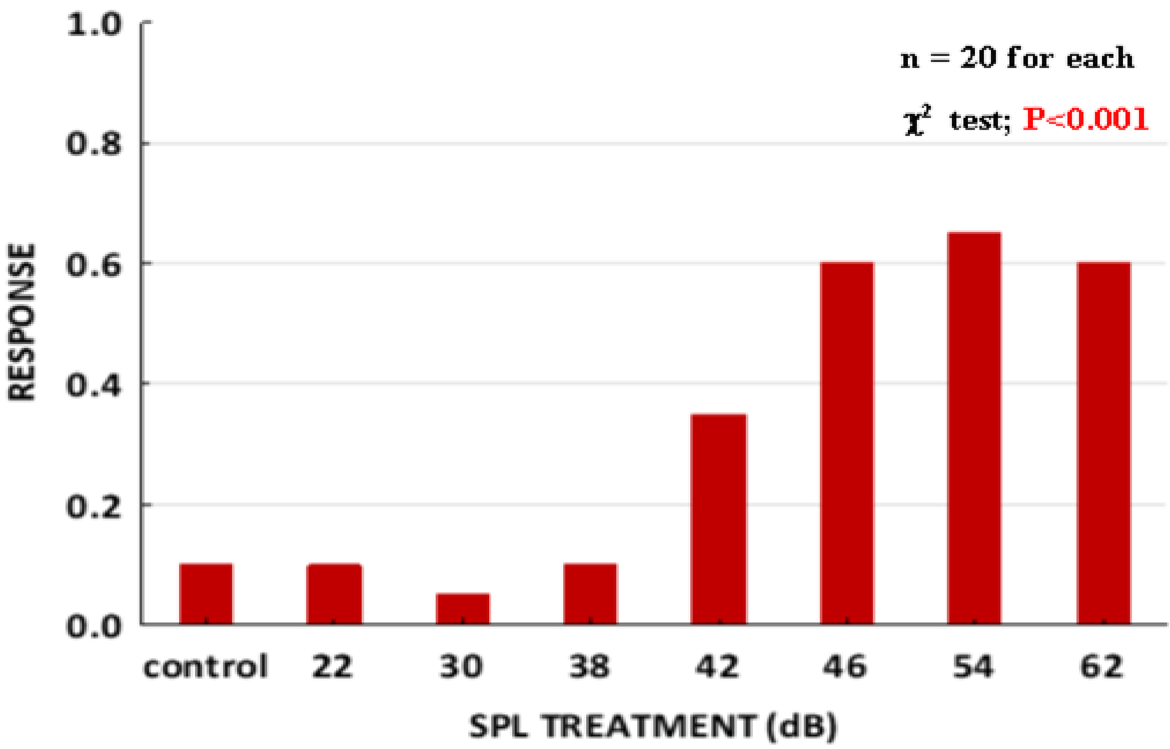

Figure 2.3.1: Proportion of response of female towards different SPLs under ambient noise condition (9 dB to 11 dB (±0.89)). Each bars indicates the relative proportions of positive phonotaxis responses

Behavioural hearing threshold under traffic noise condition

Out of four experimental trials, only 68 dB showed significantly higher positive responses than the negative control which is having no call stimulus but only traffic noise (Chi-square (df=1)=12.13, P= 0.0005). 46 dB, 54 dB and 62 dB did not show any statistical difference with the negative control (Chi-square (df=1) =.14, P= 0.7050; Chi-square (df=1) =1.90, P= 0.1676; Chi-square (df=1) =2.85, P= 0.0914 respectively).

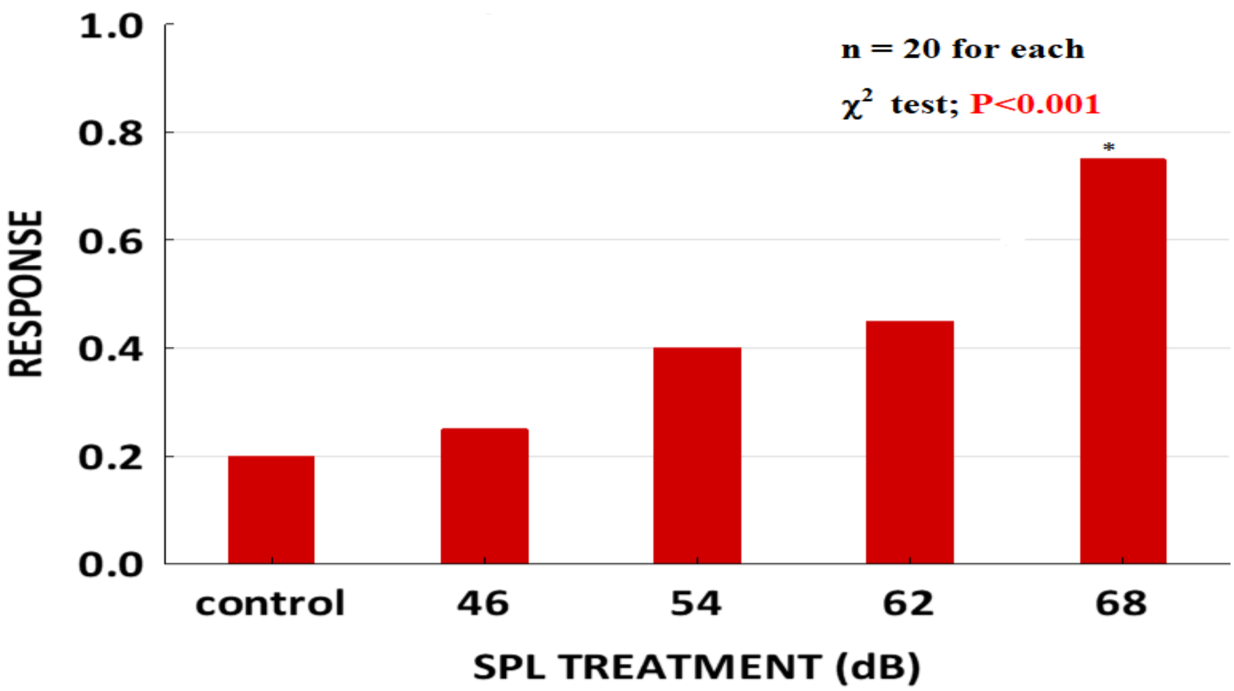

Fig 2.3.2: Proportion of responses of female towards different SPLs under traffic noise condition (55 dB at 5 kHz). Each bars indicates the relative proportions of positive phonotaxis responses

Discussion

The results of this study document the influence of loudness and noise on signal detection. The behavioural hearing threshold for *A. asiaticus* was found to be 46 dB under quiet conditions (10 dB at 5kHz, 40 dB at broadband) whereas it shifted steeply upwards to 68 dB in the presence of traffic noise (55 dB at 5kHz and 74 dB at broadband). These results clearly show the impact of noise on the behavioural hearing threshold of the *A. asiaticus* females. It also shows the constraint created by traffic noise in their audible range. The shift in the BHT can be considered as TTS (temporary threshold shift) but further examination is needed for understanding the persistence of this shift.

All the results were obtained under controlled lab conditions but the noise intensity and temperature were set to mimic the natural habitat. Unlike the open-loop methods, the study followed a no-choice arena setup (closed-loop method) where the animal is getting active feedback in relation to the sound source. This also can make the setup and results further similar to a natural setup.

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3. How much louder must the male be to be chosen? Female mate-choice in relation to call amplitude

Advertising for a potential mate is a well-known phenomenon in the animal kingdom. Advertising strategies can vary from one species to another in numerous ways. The vivid plumage of the peacock and the complex vocalisation of the song sparrow differ in their communication modality but serve the same function of mate attraction. In particular, acoustic signals can travel over long distances which allows for efficient communication (Gerhardt and Huber 2002; Bradbury and Vehrencamp 2011). Additionally, acoustic signals often serve as a substrate on which female choice can act where higher quality signals have characteristic properties and are preferred (Popov *et al.* 1974, Thorson *et at.* 1982, Huber *et al.* 1989).

In field crickets, females prefer more energetic signallers who are capable of advertising it during signal production (Ryan & Keddy-Hector, 1992). From the literature, it has been revealed that variation in parameters such as loudness (Forrest. 1983; Pacheco and Bertram 2014), chirp rate (Wagner 1996; Wagner and Reiser 2000) and carrier frequency (Prestwich 1996; Gerhardt and Huber 2002) can influence the probability of a male being chosen by the females. This can be explained as the correlation of male quality, in other words, the call features can be a proxy for greater material benefits (e.g., Gwynne 1982) and genetic quality (e.g., Simmons 1986, 1987) of the signaler. The intensity of the call is the main proxy for females to recognise better quality signallers. This can be observed in mole crickets where intensity is an indicator of body size (Forrest 1983). Putting aside the inherent advantage of having larger broadcast areas and increasing the probability of getting detected by the females, louder signals are known to be preferred by the females (Pacheco and Bertram 2014). If the females prefer louder signallers over softer ones, these louder individuals may gain an advantage in terms of female preference in addition to higher detectability.

Examining the female mate choice based upon the loudness may provide us information about the evolution of signaller's intensity of call. It is also important to see what difference in loudness will yield to a preferential response. Related questions such as what differences in loudness of two calls will elicit a choice in females and whether at all one call is more attractive over others if it lies in a softer region of population range of sound pressure level has been tested before by Pacheco and Bertram (2014).

Objectives

1. To examine whether louder long-distance mating calls (LDMC) provides an advantage for male *A. asiaticus* during mate attraction
2. To examine whether the advantage is limited by a minimum threshold difference in sound pressure levels between two signals

Methodology

Rearing and maintenance of culture

The cricket culture was maintained under standard and stable lab conditions. Details have been provided in chapter 2. Crickets with age of at least 1 week after final moulting were used for the experiments.

Playback set-up and settings

The playback experiments were conducted in a phonotaxis arena in a two-choice paradigm. The call stimulus (having temporal and spectral parameters reflecting the population average) was taken from a library of cricket calls recorded from the field (Singh and Jain unpublished). One of the two speakers transmitted a loud stimulus whereas the other played the softer call. SPL combinations of 46dB vs 49dB, 46dB vs 52dB were tested to find the choice of females during mate finding. The stimuli were transmitted with minimal overlapping of chirps. This was achieved by alternating the two signals in phase. Two speakers (kept 1m apart from each other) were placed 1m away from the point of release at an angle. These speakers were switched between left and right positions in order to control for the side bias. The SPL of the calls were measured using a Bruel & Kjaer ½'' microphone, Type 4189 (20 Hz to 20 kHz) which is attached to a Sound Pressure Level Meter, Type 2730 (Bruel & Kjaer, Naerum, Denmark) at a distance of 0.5 m from the active speakers. The test stimuli pairs which differed in SPL, (46 dB - 49 dB and 46 dB) were played back, starting from the softest to the loudest from one of the speakers placed in the arena. This was done because of the possible interference of louder calls in animal's behaviour if those calls were played at the beginning. Each playback trial lasted for 5 minutes.

Phonotaxis trials (two-choice paradigm)

Phonotaxis trials were carried out in a similar way as described in chapter 2 except that in this case both the speakers were active, thereby making it a 'two-choice paradigm'. A given female was tested not more than once per night.

Arena

The phonotaxis arena was the same as the one used for testing BHT (Fig 3.1) with the only difference being that both speakers were active.

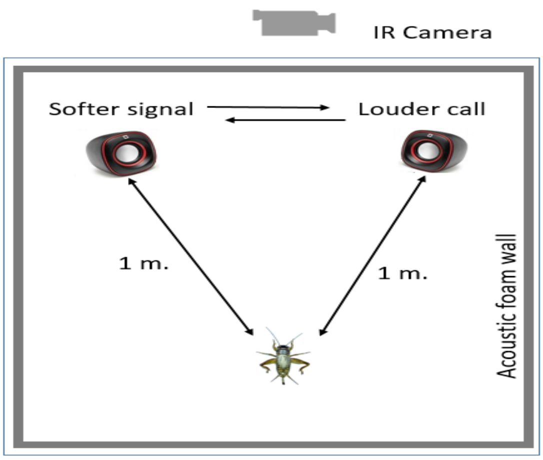

Figure3.1: Representation of a two-choice arena

Experimental protocol

Adult *A. asiaticus* female crickets were isolated socially and acoustically from others. These crickets were tested in phonotaxis experiments in a two-choice paradigm. A total of

15 individuals were exposed to the 46dB-49dB trial whereas 16 individuals were exposed to the 46dB-52dB trial. Experiments were carried out from 7 pm to 11 pm (peak activity time of the crickets). One female was tested only once per night. After every trial, the sides of stimuli exposure were swapped to control for side bias and the soil was shuffled to remove the olfactory cues. If the female was not responding, we checked for female motivation by exposing it to a call of an average SPL (62dB). The choice of the individual, the latency of the response, loudness of the stimuli and the side of exposure was recorded in a datasheet and the trial was recorded in MTS format using an infrared camera (Canon model-XA20, Japan). The response data was later on tested using statistical analyses software.

Statistical analysis

All the statistical analyses were done using Statistica (64 bit, version 12.7.207.0). A female's preference/choice towards either one of the experimental stimulus was recorded as a response or no response. This data was statistically tested using χ^2 p-value test. The stimulus which has a significantly higher response compared to the other was considered as the preferred call during phonotaxis.

Results

Both of the two-choice trials were analysed and it was observed that the phonotactic trials with calls of 3 dB difference in loudness (46dB vs 49dB) showed no statistical difference (Chi-square (df=1)=1.22 P= 0.2690) whereas trials with calls of 6 dB difference (46dB vs 52dB) showed a significantly higher response towards the louder call (52 dB). Although calls with 3 dB differences weren't statistically different, it had a slight preference towards the louder call.

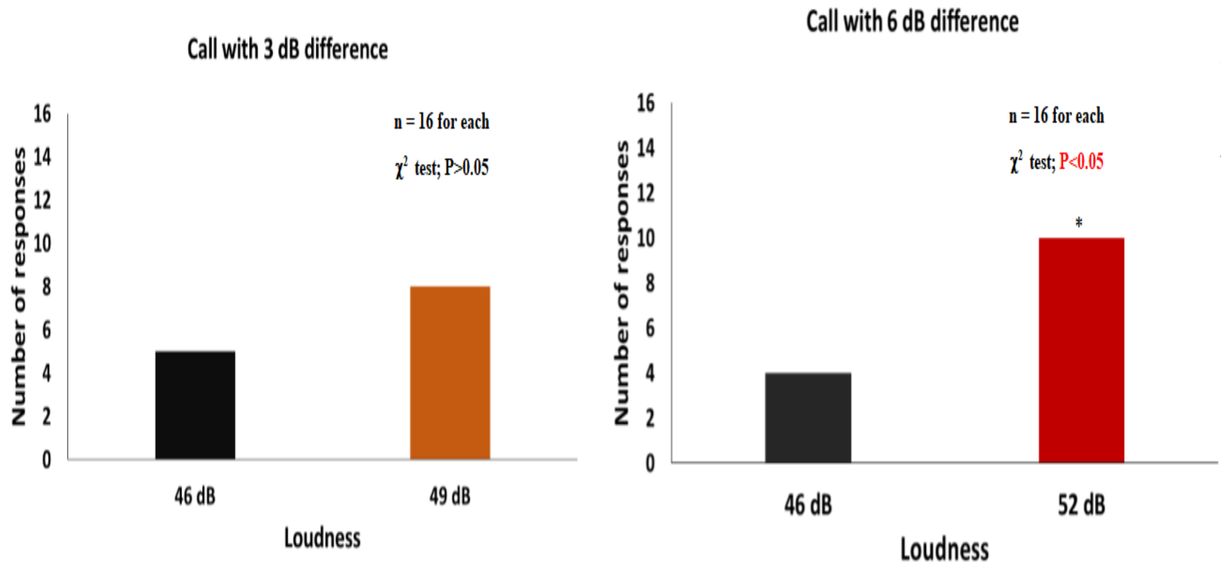

Figure3.2: The responses towards differentially loud stimuli. Females chose 6 dB louder call more than the softer call. 46dB-52dB: left, 46dB-49dB: right.

Discussion

The results clearly suggest a preference of females towards louder call, but only when the difference between the louder and softer call was 6 dB. These findings are similar to those from a study done by Hirtenlehner and Römer (2014) on female field crickets (*Gryllus bimaculatus*) under natural outdoor conditions where the threshold difference in loudness was 5 dB for discriminating between the calls.

However, there seems to be a lower limit to the difference in loudness between the calls, below which the preferential response to the louder calls ceases to exist. Here, we have seen that a 6 dB difference in the loudness is sufficient to elicit a preferential response, but there is a statistically insignificant preference towards the louder call when we expose them with calls having a 3 dB difference only. This study with two-choice arena setup points towards a loudness difference of 6 dB as a requirement for decision making. However, a 6 dB threshold of loudness is a conservative estimate and follow-up studies with loudness differences of 4 dB and 5 dB have to be done to resolve this value of threshold difference.

These results hold true only when the softer call is at BHT i.e. 46 dB. The obtained results are prone to change if one shifts this softer call away from BHT. Further studies in the system can examine whether these results hold true for loudness values other than BHT. This study shows that the field crickets, *A. asiaticus* are capable of discriminating between two relatively softer audible calls provided that these calls are sufficiently separated by their loudness.

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4. How far can the male be heard? Sound transmission in different environmental conditions

One can categorise the major ways in which the sound signal undergoes degradation during its propogation; Degradation of amplitude, spectral and temporal features of the signal (Forest, 1994). The major causes of degradation of these features are listed below,

Degradation of amplitude features

- Spherical spreading: The finite amount of energy which gets broadcasted from a point source will spherically spread into the atmosphere and this will, in turn, reduce the energy density at a given point. This can lead to a 6 dB loss of intensity per doubling of distance(fig 4.0) (Forest, 1994; Römer. 1998).

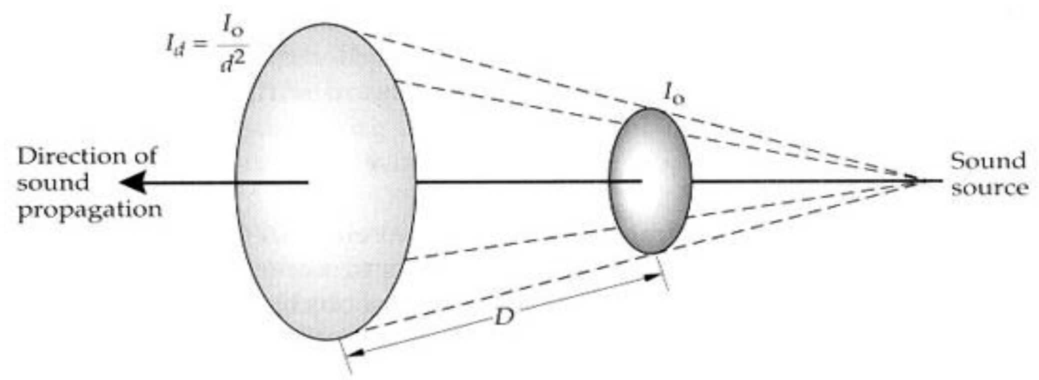

Fig 4.0: The mechanics of Spherical spreading. Shows how the energy distribution is happening with distance. (Bradbury and Vehrencamp 1998)

- Excess attenuation: Excess attenuation can be defined as the additional attenuation imposed on the sound signal other than spherical spreading at a given point (Forest, 1994). This can happen as a result multiple environmental factors. One of it is atmospheric absorption and scattering due to obstacles in the path (Forest, 1994) where the propagated signal loses its energy in the form of heat loss due to the relaxation of molecules (Bass, 1991). The other major ways in which excess attenuation happens is scattering (where the sound scatters during the transmission through medium) and ground effects (interaction of emitted signal with the ground) (Forest, 1994). Animals which broadcasts signal near ground such as insects and anurans faces ground effects severely than others (Forest, 1994). The sound propagation near ground can be influenced by the arraignment of signaller and receiver specially, the signal wavelength and the acoustic impedance of the ground (Embleton *et al.* 1983)

Degradation of spectral features

- Signal-to-Noise ratio: Noise can influence the degradation of spectral features of a sound singnal (Ryan and Brenowitz, 1985; Romer *et al.* 1989). In chapter 2 we have discussed about the different forms of noises which is categorised into biophony, geophony, and anthrophony (Bryan *et al*. 2011). Masking of a particular signal will happen when frequency spectrum of the noise and of the signal overlap. The proportion of this overlap determines the quality of the signal and one can take this as a quantitative measure of signal quality which is signal-to-noise ratio (Ryan and Brenowitz, 1985; Romer *et al.* 1989). In the context of growing urbanisation happening all around the globe, anthropogenic noise or anthrophony is one the main sources of spectral degradation of acoustic signals of animals living in urban environments (Gurule-Small and Tinghitella. 2018).
- Absorption: Low frequency sound signals are capable of travelling longer distances than high frequency sound due to the absorption happening in the medium of sound propagation. The medium absorb the acoustic energy in the form of heat energy during the relaxation of vibrating molecules (Bass, 1991)
- Environmental filtering: The studies carried out by Morton in 1975 revealed a phenomenon in which the environment filters particular ‘windows of sound’ or frequency bands more than other frequency bands. A series of follow-up studies suggests that the sound window which is getting filtered out from an acoustic signal is highly depended upon the characteristics of that habitat (Marten et al. 1977; Marten and Marler, 1977; Waser and Waser, 1977)

Degradation of temporal features

- Multiple paths: The acoustic signal may go through reflection (echoes) during transmission and these echoes may constructively or destructively interfere with the signal itself because of the phase difference between them at receiver point. This could lead to the destruction of temporal signatures of the call (Forest, 1994; Römer. 1998).
- Multiple sources: In the presence of more than one signaller, signalling nearby will lead to the interference of these signals which in turn alter the temporal structure of the signal (Forest, 1994; Römer. 1998)

The above literature on various sources of signal degradation is suggesting different transmission ranges in different environmental conditions (Except in the case of spherical spreading). One could possibly examine the combined effect of these factors in signalling in various habitats. In one habitat, for a signal to get detected, it should reach the receiver with at least a loudness which is higher than the hearing threshold of the animal (Huber *et al.* 1989). Therefor it is essential to understand how the signal attenuation is happening in different habitats.

The expanding nature of anthropogenic noise due to rapid urbanisation could also affect the signal transmission by masking signals with similar spectral features (Gurule-Small and Tinghitella 2018). There for examining the signal attenuation along with masking by anthropogenic noise could reveal possible impact of anthropogenic noise upon animals which are living in urban environments.

Objectives

1. To characterise sound transmission of *A. asiaticus* LDMC in three different calling sites.
2. To extrapolate the data for traffic prone regions.

Methodology

Study sites

Three sites were chosen differing in their ground foliage density inside IISER Mohali campus for conducting the transmission experiments. The first area (fig 4.1 A) where we conducted the study was shaded and covered with leaf litter. The second one was an open area with grassy ground (fig 4.1 B) and the third study site was also an open field with no vegetation on the ground(fig4.1 C).

Figure 4.1: three sites where the transmission experiments were conducted. A- Area near to shopping complex (30°39'50.50"N, 76°43'36.89"E), B- Area near to the football stadium (30°39'54.18"N, 76°43'29.73"E), C- Area near to the visitor's hostel (30°39'47.00"N, 76°43'24.53"E)

Experimental protocol

The transmission of *A. asiaticus* long-distance mating calls was tested up to 32 m in each habitat. The call with average parameters was played back from a speaker at point A. Loudness was set at 62 dB at 0.5m. Three consecutive SPLs were taken at distances 1m, 2m, 3m, 4m, 5m, 6m, 7m, 8m, 9m, 10m, 16m and 32m. The noise was measured using the SPL meter at 5KHz. All these sounds were recorded using a TASCAM recorder. Humidity and temperature were measured using a KESTREL weather meter.

Fig 4.2: measuring SPL during the experiment

Statistical analysis

The average of 3 consecutive SPL values was calculated for every distance. These average SPL values were plotted against distance for each habitat. The average SPL for the three habitats was also plotted against the distance using MS Office Excel. The SPL of day time noise at 5 kHz, night time noise at 5 kHz (Singh and Jain unpublished), and the traffic noise at 5 kHz were also plotted in the same graph. The distance corresponding to the intersection of sound transmission and the noise level was considered as the maximum transmission range.

Results

The sound transmitted more efficiently in habitat A whereas the rest of the habitats were comparatively poorer in transmission. Average transmission range was found to be 9 m. at day time noise condition (25.66 dB at 5 kHz), 4 m. at night time noise conditions (38 dB at 5 kHz), and 1 m. at traffic noise condition (55 dB at 5 kHz, see fig 4.4).

Habitats	Maximum transmission range without getting masked (dB)		
	Day time noise (26 dB, 5 kHz)	Night time noise (38 dB 5 kHz)	Traffic noise (55 dB, 5 kHz)
Habitat A	9	5	1
Habitat B	6	2	.5
Habitat C	7	3	.5

Table 4.3: Habitat-wise transmission ranges

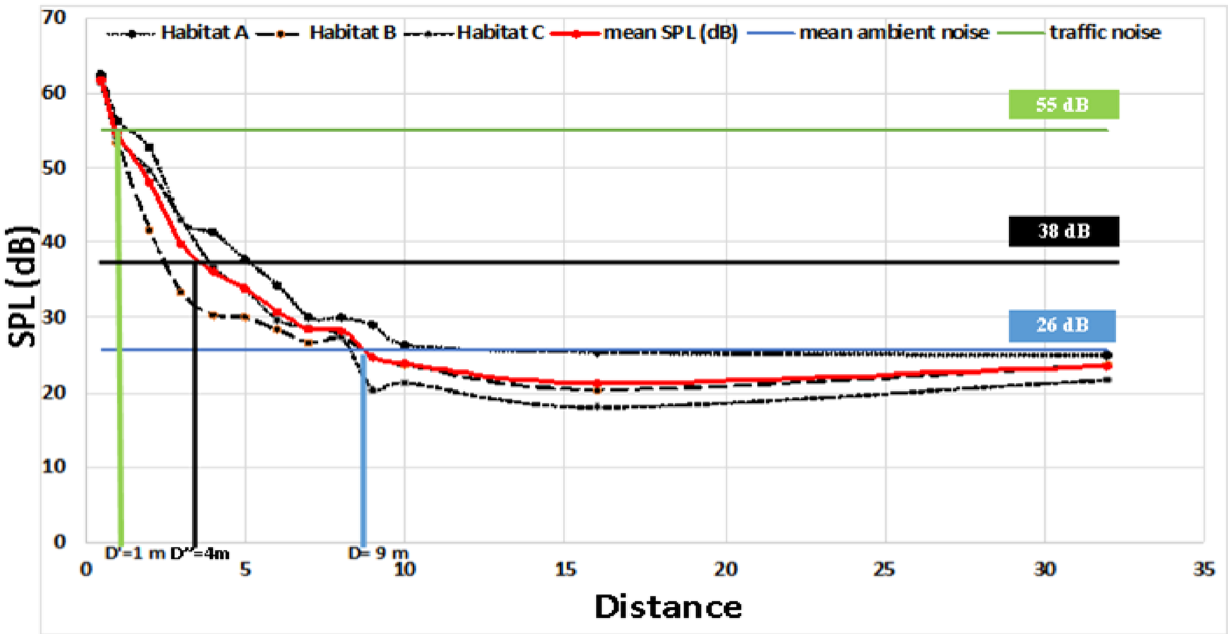

Fig 4.4: Transmission of cricket sound along with different noise conditions. What do the four different lines stand for? Point of intersection of average transmission and noise floor has taken as maximum transmission range.

Discussion

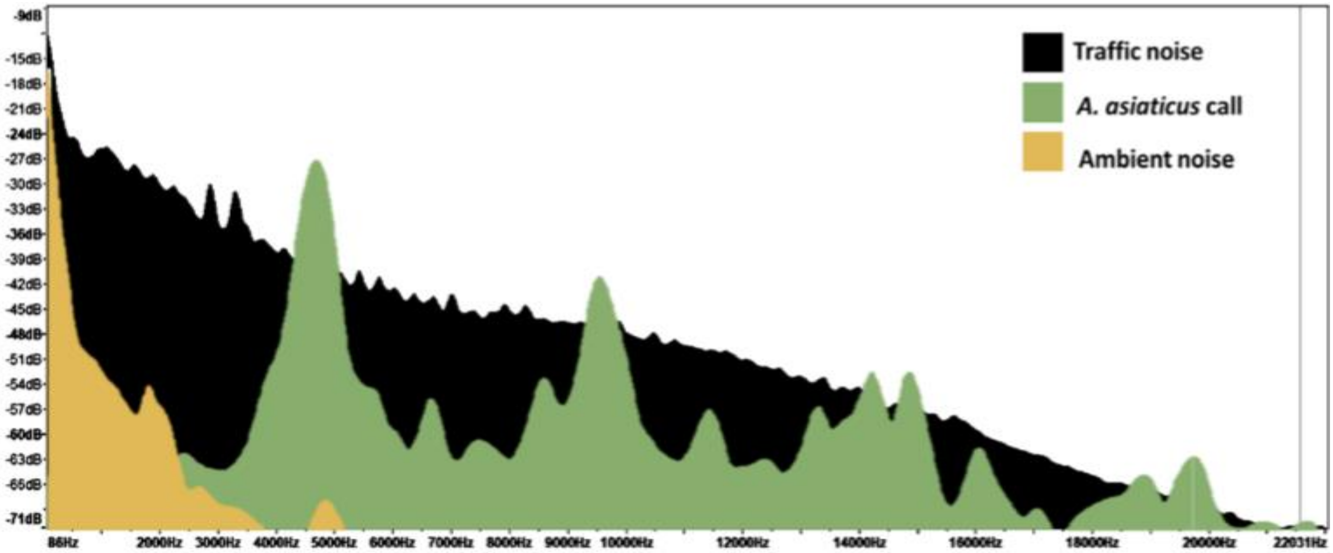

Fig 4.5: Spectral representation of *A. asiaticus* LDMC with average acoustic parameters under different noise conditions

The results show differences in maximum transmission range due to the dissimilarities in the environmental factors present in each of the habitats. Even if one normalises these factors, the ambient noise present at that habitat is capable of limiting the effective range of transmission. In other words, habitat-induced signal degradation is not the sole reason for limiting transmission range, masking of signal due to elevated noise conditions plays an important role in determining this. Therefore, a male which is calling in a traffic prone area has to deal with both habitat-induced transmission loss and masking due to elevated noise floor.

Apart from the difficulties faced by signallers in transmitting the signal, we have already observed an increased BHT for the receivers which are exposed to traffic noise condition (refer chapter 2). The impact of anthropogenic traffic noise on both signaller and receiver could severely interfere with the acoustic communication happening between male and female crickets.

It would be interesting to see how populations inhabiting similar habitats mitigate this issue. One solution for the signaller is to produce louder calls to increase the maximum transmission range which makes a direct trade-off with energy allocation for signal production (Hoback and Wagner 1997). Male grasshoppers (Lampe, Reinhold, & Schmoll, 2014), birds (Slabbekoorn & Peet, 2003, Slabbekoorn, 2013) and anurans (Cunnington & Fahrig, 2013) show a shift in their dominant frequency to avoid spectral masking in urban environments. Yet solutions for receivers to overcome the same problem isn't so trivial. This area remains largely neglected (Lugli 2018) and has scope for further exploration. A recent study by Lugli (2018) tried to model the hearing sensitivity curve in response to ambient noise conditions.

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5. Conclusions and future directions

BHT of females in quiet and noisy conditions

The major findings of this study on loudness reveal that the BHT drastically shifted from 46 dB to 68 dB when the traffic noise was introduced into the ambient noise conditions which clearly shows the effect of elevated anthropogenic noise on the signal detection ability of female crickets. However, it is necessary to examine the threshold under intermediate noise levels to see this trend in shifting of BHT. Detailed follow-up studies need to be carried out to examine whether the shift in BHT is governed by the change in signal-to-noise ratio, the acoustic characteristics of the noise itself, their interaction or other parameters.

Female mate-choice in relation to call amplitude

The phonotaxis experiments in the two-choice paradigm showed an elevated response rate towards the louder call. The threshold of signal discrimination for female choice was found to be 6 dB under ambient noise conditions but this threshold value has to be resolved between 3 dB and 6 dB by conducting more trials with calls with 4 dB and 5 dB loudness difference. Examination of this preference throughout the audible range of the female crickets has to be done in order to understand whether it is changing with the absolute loudness of these exposed calls.

Sound transmission in natural habitat

The transmission experiments were helpful in understanding the the distance over which sound transmits in the natural habitat of the animal. It also highlights that in different sites the transmission profiles were not identifical. Further, by applying different noise floors the study shows that the effective transmission range reduced from 4 m to 1 m when the noise floor was changed from quiet night time conditions to traffic noise conditions. Further studies to estimate the active spacing and population loudness of mating calls in these conditions will provide us more data to support this reduction in transmission range.

Future directions

The prime objective of the study was to look at the effect of loudness on female mate preference and to examine the impact of traffic noise in signal perception and propagation. It will be equally interesting to investigate the role of other temporal and spectral parameters such as chirp rate and carrier frequency in driving the female mate preference. This might provide insight into the combinatorial influence of these parameters in signal recognition and response (Huber *et at.* 1989).

As a response to growing urbanisation events happening around the world, it is highly relevant to look at the impact of noise pollution, a by-product of urbanisation, in order to see its impact on the physiology and behaviour of different life forms. The ease of handling and maintaining them crickets in lab and the suitability to carry-out controlled lab experiments make crickets a highly feasible model system to explore the influence of anthropogenic noise in acoustic communication. Therefore, it opens the scope for advanced research in this field. The evolutionary strategies adopted for mitigating the damages caused by anthropogenic noise in signal propagation is an active area of research whereas the impact of it in signal perception or hearing is highly underexplored (Lugli 2018).

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