Examining the effect of varying social and acoustic environments on the courtship behaviour of a field cricket, *Acanthogryllus asiaticus*

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A dissertation submitted for the partial fulfilment of

BS-MS dual degree in Science

Indian Institute of Science Education and Research Mohali
May 2020

CERTIFICATE OF EXAMINATION

This is to certify that the dissertation titled "Examining the effect of varying social and acoustic environment on the courtship behaviour of a field cricket, *Acanthogryllus asiaticus*" submitted by Karthik T (Reg. No. MS15170) for the partial fulfilment of BS-MS dual degree program 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 be accepted.

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Dated: May 4, 2020

DECLARATION

The work presented in this dissertation has been carried out by me under the guidance of Dr. Manjari Jain at the 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 acknowledgement of collaborative research and discussions. This thesis is a bonafide record of original work done by me and all sources listed within have been detailed in the bibliography.

Karthik T

Dated: May 4,2020

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

Dr. Manjari Jain

(Thesis Supervisor)

Acknowledgment

First and foremost, I would like to express the deepest gratitude towards my thesis advisor, Dr. Manjari Jain, for the invaluable guidance she provided throughout the course of this work. My time in her lab has equipped me with the skillset necessary to step into the world of scientific research with confidence. Without her guidance and persistent help, this thesis would not have been fruitful. I would also like to thank Dr. N.G Prasad and Dr. Rhitoban, for reviewing my thesis. I am especially indebted to IISER Mohali for infrastructural support and DST-INSPIRE for fellowship.

I am thankful to all BEL members – Dr. Sujata, Anindya, Richa, Sonam, Soniya, Nakul, Pankha, Adi, PP, Negi, and Deepak - for providing an environment where serious research and wholesome fun goes hand in hand. Discussions I had with Anindyo, Richa, and PP were crucial for the completion of my experiments. I thank Adil and Geetika for all the help they provided during experimental sessions. I am grateful to our field assistant Mr. Gurmeet Singh for helping with the collection and maintenance of crickets. Also, all those late-night lab parties provided memories that will be cherished for life.

I am blessed to have friends who stayed through thick and thin during the last five years of my time in IISER. Especially thankful to Deepu, Babu, Thabu, Nahas, Ziya, Anees, Habeeb, and Atvi for providing lasting friendship. I am thankful to Jain for all the adventures and memories. I thank Anu for always bringing a smile to my face. Finally, I am indebted to my mother for all the love and care she provided, which made me the person I am today.

Thanks again for your contribution, love, and guidance.

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Chapter 1: General introduction to the effect of social environment on behaviour: the audience effect

1.1 Costs and benefits of signaling and receiving in a network environment

Communication plays a vital role in virtually all crucial social behaviours. Communication can itself be considered as a social behaviour since at least two individuals, a signaler and a receiver, must be present for it to be facilitated (McGregor and Peake, 2000). Often, the social environment in which communication takes place is ignored in discussions of communication behaviour. With the exception of choruses, most studies dealing with communication, implicitly or explicitly, generally only consider the dyadic aspect of communication.

All communication takes place in a network environment – except when the signal can never be received by more than one receiver, or a receiver can never receive more than one signal simultaneously (Peake, 2005). A network of several signalers and receivers constitute the social environment in which much of the communication occurs (McGregor and Peake, 2000). A signal that transmits over long-ranges will encompass multiple individuals during the course of transmission. This area encompassed is sometimes referred to as the signal's active space (Brenowitz, 1982). Rather than absolute distance, the distance travelled by the signal in relation to the mean spacing between individuals is the central idea of communication networks. Considering many social groupings of animals in the wild are adjacently spaced, signals that are short-range in an absolute sense can contain multiple signallers and receivers. Thus, communication network is the most prevalent social environment in which communication takes place (Dabelsteen,1992; McGregor,1993; McGregor and Dabelsteen,1996). This type of network environment imposes additional selection pressure on both signallers and receivers other than those classically considered in signaller-receiver dyads.

1.1.1 Receivers in a network environment

The difficulty of discriminating information from individual signallers while multiple signallers are simultaneously active is one of the costs associated with receiving signal in a network environment. For example, adult king penguins (*Aptenodytes patagonicus*) returning from foraging in the ocean to feed its chick faces such an issue of discriminating its own chick from the rest (Aubin and Lengagne, 1998; Jouventin *et al*, 1999). Echolocating bat, and electrically communicating fish share similar problems in a network environment. (e.g., Bastian, 1987; Møhl and Surlykke, 1989).

Acquiring information from signalling interactions between others, or eavesdropping (McGregor and Dablesteen. 1996), is one benefit of being a receiver in a communication network. Extracting information at little cost and no risk are few of the advantages gained by an eavesdropper in such an environment. For example, male Siamese fighting fish *(Betta splendens)* eavesdrop on the visual displays of potential future rivals and use this information collected to change their behaviour in the beginning phases of aggressive interactions (Oliveira et al. 1998). Similarly, by eavesdropping on male-male agonistic interactions, females can gather information regarding the relative quality of males and alter their mate choice (Aquiloni, 2008).

1.1.2 Signallers in a network environment

As mentioned in the above section, receiving in a network environment has associated costs and benefits. Similarly, in such an environment, signallers also face different costs and benefits. In a communication network, an individual signaller faces two main problems: (a) it has to contest or cooperate with other signallers, and (b) it has to deal with the presence of multiple receivers (Peake, 2005). Signallers mitigate the effect of former problem by adopting various strategies. As an example, in anurans and insect choruses, individuals avoid acoustic interference by timing their signals or competing for the call order in the chorus (Gerhardt and Huber, 2002). Another strategy for species with similar signal structure is to transmit their signals at different times of the day (Endler, 1992). The signaller faces two additional issues while signalling near multiple receivers: (a) directing the signal to a specific receiver (b) communicating in the presence of multiple receivers apart from the primary receiver. Audience effect falls under this second problem.

1.2 Audience effect

Audiences are individuals that are present during, but do not actively take part in, signalling interactions between others. The mere presence of an audience can alter the signalling behaviour of interacting individuals and this phenomenon is termed as audience effect (Matos and Schlupp, 2005). If costs and benefits are associated with the presence of an audience, the signaller should adjust their behaviour towards the targeted receiver in order to conceal or enhance the information they transmit (McGregor and Peake, 2000). There are broadly two different types of audiences – evolutionary audiences and apparent audiences.

Evolutionary audiences are individuals that were historically present in the environment of the signaller, and that may have generated selection on the form and content of the signalling behaviour (Matos and Schlupp, 2005). For example, one hypothesis suggests that the dual function of bird song evolved as a result of the eavesdropping pressure caused by male audiences (Searcy and Nowicki, 2000). Since selection has acted in the past and probably continues to act, the actual presence of an evolutionary audience is not needed to elicit changes in behaviour. On the other hand, apparent audiences are individuals that changes the signalling behaviour only when they are present and detected. As an example, presence of female audience significantly reduces the highly hostile behaviours and increase the intensity of conspicuous displays in interacting male Siamese fighting fishes *Betta splendens* (Doutrelant *et al*., 2001). Effects produced by apparent audiences are only triggered when they are present, unlike evolutionary audiences.

Studies that used the term ‘audience’ or ‘audience effect’ for the first time checked how presence of a conspecific affected the incident of food call and alarm call in birds (Gyger *et al*., 1986; Marler *et al*., 1986; Gyger, 1990; Evans & Marler, 1994). However, in these experiments, the distinction between a primary receiver and an audience was blurred as the target receiver itself was the audience. Since there was no additional individual present as an audience in these interactions, it does not fall under the modern definition of audience effect. Studies which followed investigated how the presence of an audience influenced the behaviour of individuals in different contexts.

In the context of aggressive interactions, the presence of an audience elicits changes in some animals (eg., Matos, 2002; Fitzimmons and Bertram, 2013). Acquisition of territories, mates, or food often requires individuals to undergo competition. Displays used by individuals during such aggressive interactions can be used to assess their motivation and fighting ability (Huntingford & Turner, 1987; Bradbury & Vehrencamp, 1998). In a communication network, apart from the opponent, this information is available to the individuals present inside the signal range. As an example, 'loser' of a male Siamese fighting fish pair, restricts the information available to a male audience by changing their display behaviour (Matos, 2002). This behaviour fits the observation that losers are more readily approached by males that saw them lose (Oliveira *et al.,* 1998; McGregor *et al.,* 2001, Early and Dugatkin, 2002). In chimpanzees, the victim of an agonistic interaction alters the acoustic structure of their scream depending on the audience's composition. Victims of severe attacks produced screams that were significantly exaggerated in relation to the aggression experienced. However, they did so only if there was at least one listener in the audience who matched or surpassed the aggressor in rank (Slocombe and Zuberbuhler., 2007).

Audience effect is also observed in the context of parental behaviour. Since there is a direct correlation between the care provided to the young and their survival until reproductive age, choosing a good male as a potential mate is advantageous to the female. Observing male interaction with the young is one way of assessing the quality of parental care by females (Matos and Schlupp, 2005). In Vervet monkeys (*Cercopithecus aethiops*), males are sensitive to the presence of the mother and engaged in less-antagonistic and more affectionate behaviour towards the infant when the male was able to detect the presence of the mother (Hector *et al.,* 1989). In Budgerigars (*Melopsittacus undulatus*), extra-pair copulation by the male significantly increased when their female mate was out of their view - suggesting an audience effect on the male's extra-pair behaviour (Baltz and Clark, 1994).

Thus, the presence and type of audience can have significant effects on the signalling behaviour of individuals. Depending on the type of audience and the role of each signaller during an interaction, the nature of information and the extent of its broadcast can vary. Audiences may also affect signal evolution as they can act as drivers of selection pressure to potentially bring about the evolution of different signals for private and public information transfer (Matos and Schlupp, 2005; Dabelsteen, 2005)

1.3 Summary of literature review

- Communication in nature more often occur within a broad social network rather than in signaller-receiver dyads.
- The presence of additional individuals in the network environment can impose additional costs or benefits on both signaller and receiver.
- Audiences are individuals that are present during but do not take part in signalling interactions between others. The mere presence of an audience may alter the signalling behaviour of individuals.
- Audiences may also affect signal evolution as they can act as proxies for selective force to potentially drive the evolution of different signals for private and public information transfer.

1.4 Crickets as a model system for studying audience effect

1.4.1 Introduction to crickets:

Crickets are nocturnal insects that use acoustics as their predominant mode of communication. Male crickets produce chirping sounds by rubbing together specialized regions of the forewings – a process called stridulation (e.g. Ewing, 1989). Both forewings possess a ventrally modified vein with a series of rigid spikes that form the stridulatory file, while the anal region of the wing harbours a plectrum. During stridulation, as the plectrum on the left forewing is swept across the file of the opposite wing, a series of impacts occur, generating vibrations of the surrounding wing membranes (Pierce, 1948). Even though the file and plectrum are present on both wings, in crickets, the right-wing usually lies on top of the left-wing (Elliott and Koch, 1985). This, in turn leads to functionally asymmetrical sound production, with the two wings having dissimilar functions (Forrest, 1987).

Different types of acoustic signals are produced by crickets:

1) Long-distance mating call (Wagner 1995) – Males use this song to attract females who respond via phonotaxis. Mate selection is possible at the level of LDMC (Doherty and Hoy, 1985) or at the stage of courtship (Brown and Gwynne, 1997).

2) Courtship call (Alexander, 1962) – courtship calls, usually softer than LDMC, are initiated by the male when it detects females in the near vicinity. Females are observed to mate only with males capable of making courtship calls (Balakrishnan and Pollack, 1996; Crankshaw, 1979; Boake, 1983)
3) Postcopulatory call (Alexander, 1962) – produced by males after copulation.
4) Aggressive song (Alexander, 1961) – produced by males during and after an agonistic interaction.

1.4.2 Acoustic characteristics of cricket call

Cricket call comprises of a collection of sound pulses, each pulse known as a chirp. Further, each chirp is made up of multiple syllables. Different parameters can be used to characterise the call structurally.

Spectral Parameters:

- Peak Frequency: frequency produced with maximum amplitude.

Sound Pressure Level (SPL): loudness of the call measured in dB.

Temporal Parameters:

- Chirp duration: Onset of one chirp to its offset.
- Chirp Period: onset of one chirp to the onset of subsequent chirp.
- Syllable duration: onset of one syllable to its offset.
- Syllable period: onset of one syllable to the onset of subsequent chirp.

1.

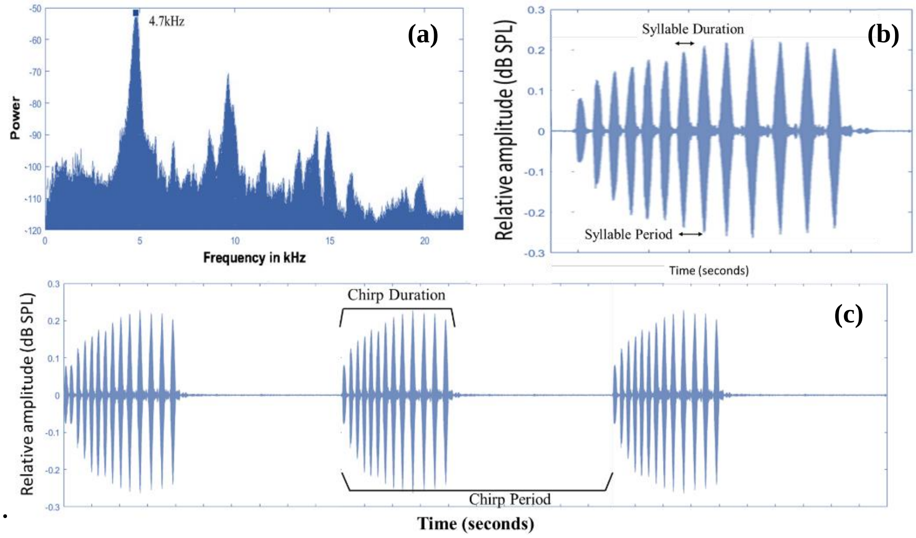

Figure1.1. Characteristics of cricket's LDMC: (a) power spectrum showing spectral features and (b), (c) Oscillogram showing temporal features. (Singh and Jain, 2020).

In field crickets, males produce a loud call when alone to attract female from a distance. Once the female makes contact, male produces a very distinct courtship call (Alexander, 1961). Courtship almost always precedes to mating, and previous studies have shown female crickets exhibit mating preferences dependent on the male courtship song (Wagner and Reiser, 2000)

Courtship experiments on field cricket *Gryllus bimaculatus* revealed three different stages of mating behaviour (Adamo and Hoy, 1994). The first stage begins with antennal contact and ends when male begins to stridulate the courtship song. The following stage starts with the courtship song and ends when female begins to follow the male. The last stage includes both female mounting and spermatophore transfer. Despite this stereotypical succession of stages, the sequence of behaviour during courtship can vary among pairs.

Visual and chemotactile contact alone usually is insufficient to elicit mating behaviour in crickets as anaesthetized females are often ignored by sexually receptive *G. bimaculatus* (Adamo and Hoy, 1994). Importance of courtship call on the mating behaviour shows conflicting results among different species. In some species, like *A. domesticus* and *T. oceanicus*, females would not mate unless stimulated by courtship song, indicating the effect of courtship call on lowering the threshold of mounting latency (Crankshaw, 1979; Burk, 1983). Whereas, experiments on *T. commodus* suggests that courtship song may be less important in releasing mounting behaviour (Loher and Rence, 1978).

1.4.4 Crickets as a model system for studying audience effect

The ubiquity of audience effect is still unknown since most of the studies examining the effect of social environment on behaviour primarily focused on vertebrates. Few published studies on audience effect in crickets provide evidence that the ability to detect audience and regulate behaviour is not limited to vertebrates. Given that cricket densities can be high, and aggressive interactions and mate attractions happen in close proximity, contests and courtships are likely to occur with the presence of male and female audiences nearby (Alexander, 1961; Ritz and Kohler, 2007; Munoz, 2011).

A study on how the presence of female influences the aggressive behaviour between males in *Gryllus bimaculatus* observed that the males increased aggressive displays towards other males when a female was present in near vicinity (Tachon *et al.*, 1999). In male *Gryllus* *veletis*, winners of agonistic interaction elevated aggressive signalling in the presence of a female audience and produced more victory displays in the presence of a male audience (Fitzimmons and Bertram, 2013). Similarly, presence of a female closer to the fight arena increased the aggressive behaviour but decreased the time spent producing victory displays of *Gryllus assimilis* (Montroy, 2016).

There is a dearth of studies investigating how the social environment affects the courtship and mating behaviour of crickets compared to studies on aggressive behaviour. A study on the striped ground cricket (*Allonemobius socius*) observed that male courtship behaviour changes according to the social environment as males were significantly more likely to skip certain courtship songs from their complex courtship call repertoire when they were in a solitary environment compared to a non-solitary environment. Moreover, female evaluation of the mates also changed when the social environment varied as mating success was higher in non-solitary environments (Sadowski, 2002)

1.5 Objectives

In light of the above discussion, I investigated the effect of social environment on cricket courtship behaviour through the scope of the following objectives.

- Quantification of courtship behaviour and courtship song in *A. asiaticus*
- Examining the effect of presence and type of an acoustic proxy for the audience on the courtship behaviour of *A. asiaticus.*
- Investigating the effect of presence and type of an audience during the courtship behaviour of *A. asiaticus*

1.6 Study species

To investigate the above-mentioned research questions, I used *Acanthogryllus asiaticus,* a field cricket species. Morphological characteristics of *A. asiaticus* (Gorochov, 1990) are:

"Body size small for genus. Head large, red along entire length and angularly bent clypeal suture, apex of angle approximately at level or lower margins of antennal pits. Colour of head dark brown, with six distinct short longitudinal pale lines on posterior part of vertex. Pronotum dark brown, with pale spots in posterolateral angles of disk. Elytra with rather transverse stridulatory ridge, more or less rounded speculum, and distinctly bent diagonal vein, area between diagonal vein and oblique veins relatively wide. Color of elytra pale brown, with dark brown stripe along upper margin of lateral area. Legs, abdomen, and cerci brownish, more or less unicolorous. Genitalia without process in middle part of posterior margin of epiphallus, with very short epiphallic apodemes, and with middle processes extending from distal half or ectoparamere and only slightly extending beyond anterior ends of ectoparameres"

Taxonomical characterisation of *A. asiaticus:*

Kingdom:	Animalia
Phylum:	Arthropoda
Class:	Insects
Order:	Orthoptera
Suborder:	Ensifera
Superfamily:	Grylloidae
Family:	Gryllidae
Genus:	Acanthogryllus
Species:	*Acanthogryllus asiaticus*

Figure 1.2 *Acanthogryllus asiaticus*: Stridulating male on the left and female with a distinguishable ovipositor on the right. (Picture credits: Richa singh)

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Chapter 2: Quantification of *A. asiaticus* courtship behaviour and courtship song

2.1 Background

In field crickets, some form of courtship ritual almost always precedes copulation. Studies on the importance of courtship call on mating behaviour show conflicting results among different cricket species – some suggest females would not mate unless stimulated by courtship song, whereas a few indicates courtship song may be less important (Crankshow, 1979; Burk, 1983; Loher and Rence, 1978). Previous courtship experiments on *Gryllus bimaculatus* revealed three distinct stages of its mating behaviour (Adamo and Hoy, 1994). Antennal contact between individuals marks the onset of the first stage. The following stage starts when the male initiates a courtship song and ends when the female begins to mount the male. The last stage includes both female mounting and spermatophore transfer.

Given that field cricket behaviour can vary considerably across species, here I examine whether *A. asiaticus* follows the same three distinct stages in their courtship ritual and quantify different courtship behaviours and courtship song in *A. asiaticus.*

2.2 Methodology

2.2.1 Collection and housing of crickets

Sub-adult crickets used for the study were collected from IISER Mohali campus (30°39′N, 76°43′E) during May-July 2019. Until their final moult into adults, sub-adults were kept in a common culture box. After final moult, adults were transferred into separate plastic containers (diameter-12 cm and height- 6 cm) containing dog food and wet cotton balls for nourishment. The temperature was maintained at 24°C.

2.2.2 Staging courtship rituals

All individuals were weighed before the experiment. Courtship pairs were not size-matched. A total of 20 male-female pairs were used for the experiment. Individuals used had no prior mating experience.

Courtship trials were staged inside a circular PVC container (height: 10Cm, diameter: 15 cm) with the base covered with soil. All trials were performed inside a dark room between 18.30 and 20.00. A removable cardboard partition separated equal areas inside the arena. At the onset of a trial, male and female crickets were introduced on either side of the partition. Both animals were given two minutes of acclimatization period before the removal of partition. On removing the partition, both audio and video recorders were turned on simultaneously. The pair were allowed to interact for five minutes. Courtship ritual was declared as finished when the female started to mount on the male and initiated spermatophore transfer. Both individuals were separated before the successful transfer of spermatophore. Before and after each treatment, the entire arena and partition were cleaned with 70% ethanol, and soil was shuffled thoroughly to make sure the elimination of any pheromones present.

2.2.3 Acoustic and camera recording

CANON XA20 infrared camera (Canon corp., Japan) and TASCAM DR-40 linear PCM recorder (DR-07 Mk II, TEAC Professional, USA), both mounted on tripods, were used for video and acoustic recording, respectively.

2.2.4 Data analysis

All the video recordings were analysed using Shotcut Video Editor. All the audio recordings were first high-pass (>2 KHz) filtered and then amplified using Audacity 2.3.1 Cross-Platform Sound Editor. Subsequently, Raven Pro 1.5.0 (Cornell Laboratory of Ornithology,Ithaca, NY) was used to analyse spectral and temporal parameters of the call. Statistica version 10 was used for Statistical analysis.

2.3 Results

Of the 20 pairs observed, one did not perform any courtship behaviour, and two failed to initiate courtship song, reducing the effective sample size to 17. Preliminary observations unravelled a discrete pattern of behaviour. Each behaviour during the courtship ritual prior to transfer of spermatophore was distinguished and tabulated (table 2.1).

In accordance with the studies done by Adamo and Hoy (1994) in *Gryllus bimaculatus*, three distinct major stages were identified in *A. asiaticus*. The first stage of courtship was observed to be initiated after the antennal contact between the individuals. First contact latency varied considerably between individuals (range: 2s – 156s) and showed a mean (±SE) of 40.5±10.7 s. Once antennal contact was established, both crickets flicked their antennae over one another, and some males immediately began courtship singing. Average courtship call latency after antennal contact was 35.7±12.6 s. Initiation of courtship singing marked the onset of stage two, where the singing male presented its rear abdomen to the female's front. If the female was receptive, she made intermittent antennal contact with the male. In some trials, the female was not receptive initially, and male performed multiple courtship song bouts (up to three times) until she finally became receptive. Average courtship call duration was 14.7±1.9 s. The mounting of female on male initiated the final stage. As the female mounted, male pushed itself back with his front legs and started to make contact with the female using its antennae by twitching them back and forth. Mean mounting latency was 53.7±11.8 s. The successful transfer of spermatophore marked the end of stage three.

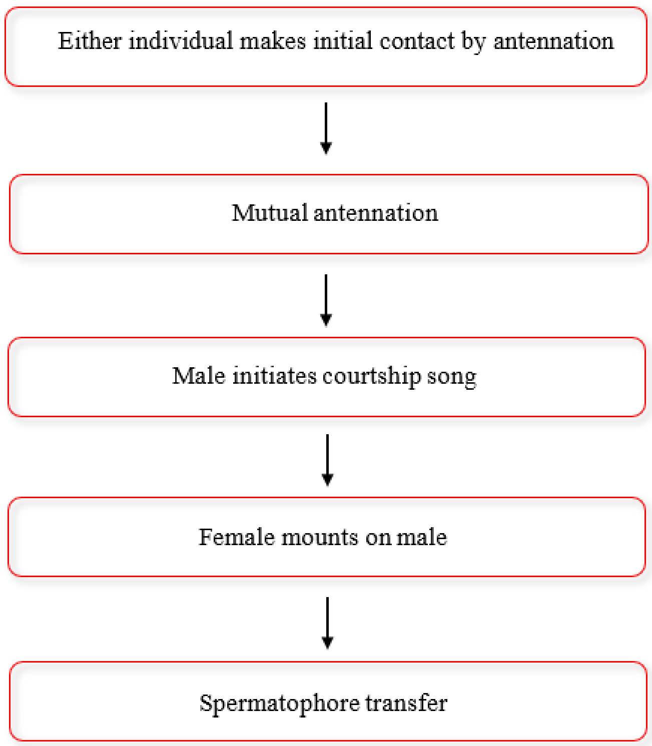

Figure 2.1. Flow chart of interactions in courtship behaviour of *A. asiaticus.*

Types of behaviour during courtship	Duration
First antennal contact latency (s)	40.5 ± 10.7
Courtship call latency (s)	35.7 ± 12.6
Total courtship call duration (s)	14.7 ± 1.9
Total antennation duration (s)	6.9 ± 1.8
Mounting latency (s)	53 .7 ± 11.8

Table 2.1. The mean (±SE) duration of different behaviours during courtship period.

Figure 2.2. Different stages of courtship interaction in *A. asiaticus:* (a) antennal contact, (b) male begins courtship singing, (c) Female initiates mounting, (d) spermatophore transfer.

(Picture courtesy: Nakul Raj)

2.3.1 Quantification of courtship call

Courtship song of *A. asiaticus* is composed of two different kinds of components with distinct spectral and temporal features – long chirps and short chirps (Singh and Jain, 2020). The loud, long chirps with an average syllable count of 8.48 ± 0.54 are followed by a continuous series of soft short chirps with an average syllable count of 3.04 ± 0.25. The average peak frequency of long and short chirps is 4.847 ± 0.056 KHz and4.607 ± 0.116 KHz respectively. Mean (±SE) values of spectral and temporal features of long and short components of courtship calls are given in the table 2.2.

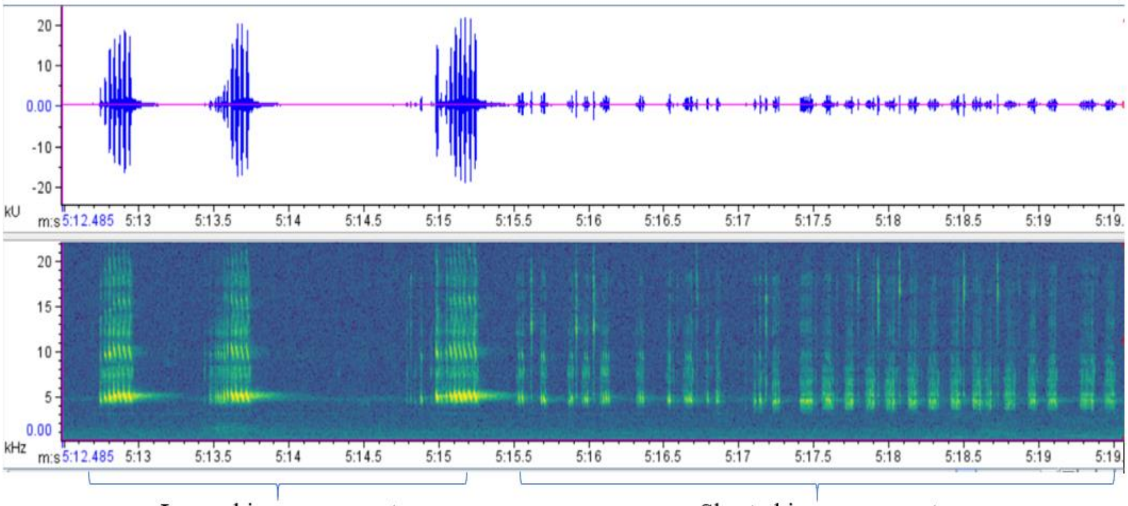

Figure 2.3. Waveform and spectrogram of long and short chirp components of courtship call.

Acoustic parameter	Long chirp of CC	Short chirp of CC
Chirp duration (s)	0.28±0.02	0.08±0.01
Chirp period (s)	2.02±0.42	0.59±0.22
Peak frequency (KHz)	4.847±0.056	4.607±0.116
Number of syllables	8.48±0.54	3.04±0.25

Table 2.2. The mean(±SE) values of different temporal and spectral parameters of courtship call long chirp and short chirp components.

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Chapter 3: To investigate the role of acoustic environment on courtship behaviour of *A. asiaticus*

3.1 Background

Since cricket densities can be high in the wild, and aggressive interactions and mate attractions happen in near proximity, contests and courtships are likely to take place with the presence of male and female audiences nearby (Alexander, 1961, Ritz and Kohler, 2007; Munoz, 2011). A handful of studies focusing on 'audience effect' in the agonistic context in crickets reveal conflicting results both supporting (Tachon *et al.,* 1999; Fitzsimmons and Bertram, 2013 and Montroy *et al.,* 2016) as well as declining (Judge *et al*., 2010) it. Studies investigating how the presence of an audience affects reproductive behaviour in crickets is even more scarce. Sadowski *et al.,* 2002 observed that male courtship behaviour of striped ground cricket changed according to the social environment.

Taking these investigations further, here, I examine how different acoustic proxy for the presence of an audience nearby affects the courtship behaviour of *A. asiaticus.*

3.2 Methodology

3.2.1 Collection and housings of crickets

Sub-adult crickets were collected from IISER Mohali campus (30°39′N, 76°43′E) from July through August 2019. Until their final moult, adults were kept in a common culture box. After final moult, adults were transferred into separate plastic boxes (to ensure social isolation) containing dog food and wet cotton balls for food and nourishment. The temperature was maintained at 24°C. One week prior to the trials, female crickets were kept isolated in a separate room to ensure acoustic isolation.

3.2.2 Staging courtship rituals

All the animals were weighed before the experiment. A total of 20 male-female pairs were used for the experiment. Only naïve males and females were used. Females were acoustically isolated for one week prior to the experiment. Silent control, LDMC playback and aggressive call playback were the three different treatments given to the focal pair. Forty-eight hours rest was given between treatments for the same focal pair. Treatment order was randomized with the help of a MATLAB (2019) random number matrix generating code.

Courtships were staged inside a circular PVC container (height: 11cm, diameter: 15cm) with the base covered with soil. A removable cardboard partition separated two equal areas inside the arena where the focal animals were introduced. At the onset of each trial, the focal pair was introduced on either side of the partition. They were given two minutes of acclimatization period before removal of the partition. This was given to familiarize the novel environment to test animals. Respective playback treatment was also given at the onset of acclimatization period. A speaker, kept 50 cm away from the arena, was used for playing back treatment calls. Upon removal of partition, the audio and video recorders were turned on simultaneously. Playback treatment was stopped when the test animals made their first antennation. The courtship ritual was declared to be concluded when the female finally mounted the male and male initiated spermatophore transfer. Before the transfer of spermatophore, the individuals were separated. Before and after each treatment, the entire arena was cleaned using 70% ethanol, and the soil was shuffled in order to eliminate odour cues.

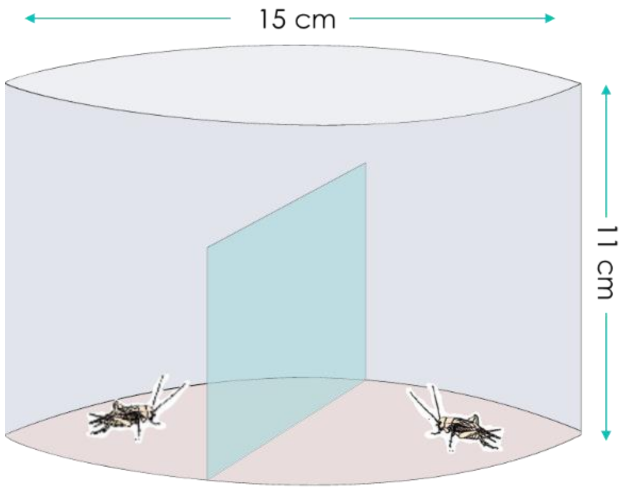


Figure 3.1. Schematic representation of the courtship arena

3.2.3 Acoustic playback

Different treatment calls - long-distance mating call (LDMC) and aggressive call of *Acanthogryllus asiaticus* - were played using a JBL Go2 speaker (JBL corp., USA) mounted on a tripod 50 cm away from the arena. The loudness of the call inside the arena (50 cm away from the speaker) was measured using a Brüel & Kjær ½" microphone, Type 4189 (20Hz to 20kHz) fixed to a Sound Pressure Level Meter, (Brüel & Kjær, Naerum, Denmark)

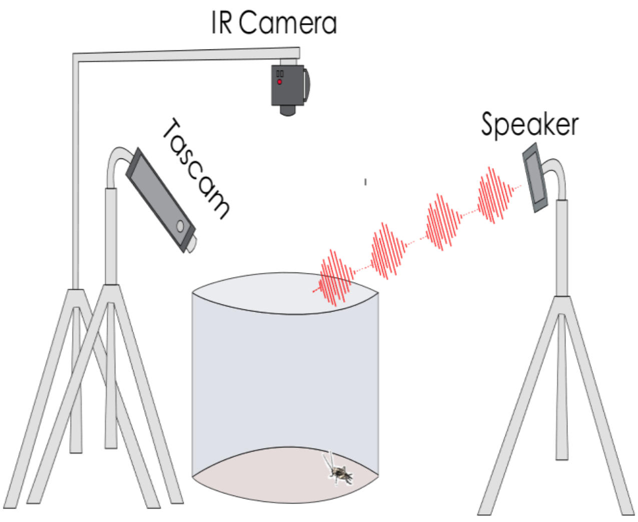


Figure 3.2. Schematic representation of the experimental setup

3.2.4 Acoustic and camera recording

To record one complete experimental session, CANON XA20 infrared camera (Canon corp., Japan) mounted on a tripod was used. TASCAM DR-40 linear PCM recorder (DR-07 Mk II, TEAC Professional, USA) mounted on a tripod was used for an acoustic recording of courtship calls.

3.2.5 Data analysis

Shotcut Video Editor version 19.08.16 was used to analyse all the video recordings. All the audio recordings were first high-pass filtered (>2000Hz) and then amplified using Audacity 2.3.1 Cross-Platform Sound Editor. Later, Raven Pro 1.5.0 (Cornell Laboratory of Ornithology,Ithaca, NY) was used to analyse various temporal and spectral parameters of the call.

3.2.6 Analysis of behaviour and acoustics

The following behavioural parameters were analysed:

- First contact latency – Time taken from the initiation of trial till the first antennal contact between focal individuals.
- Courtship call latency – Time taken from the first antennal contact till the initiation of courtship call.
- Total Courtship call duration – summation of the duration of all the individual courtship call bouts.
- Total antennation duration – summation of the duration of all the individual antennation bouts between the pair.
- Mounting latency – Time it takes from the beginning of courtship call till the initiation of mounting and spermatophore transfer.

Following acoustic parameters were analysed:

Courtship call of *Acanthogryllus asiaticus* contains both long components and short components. So, the following acoustic parameters were measured for both the long as well as the short component.

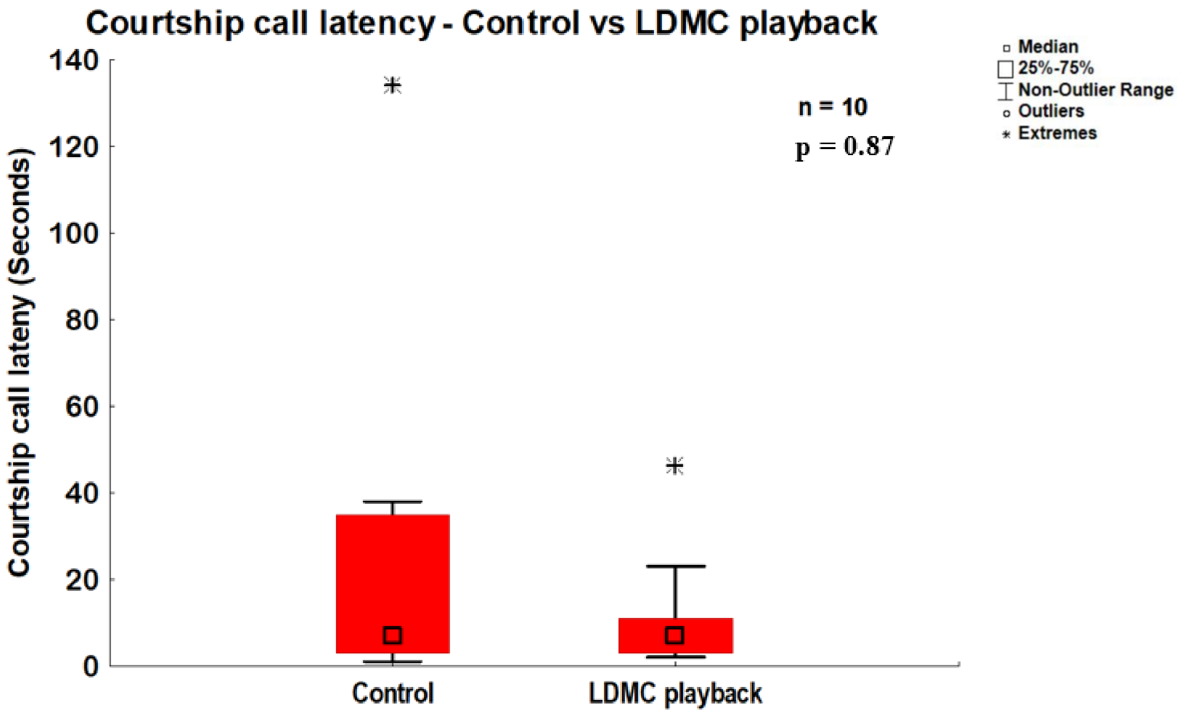
- Average chirp duration
- Average chirp period
- Average peak frequency

3.2.7 Statistical analysis

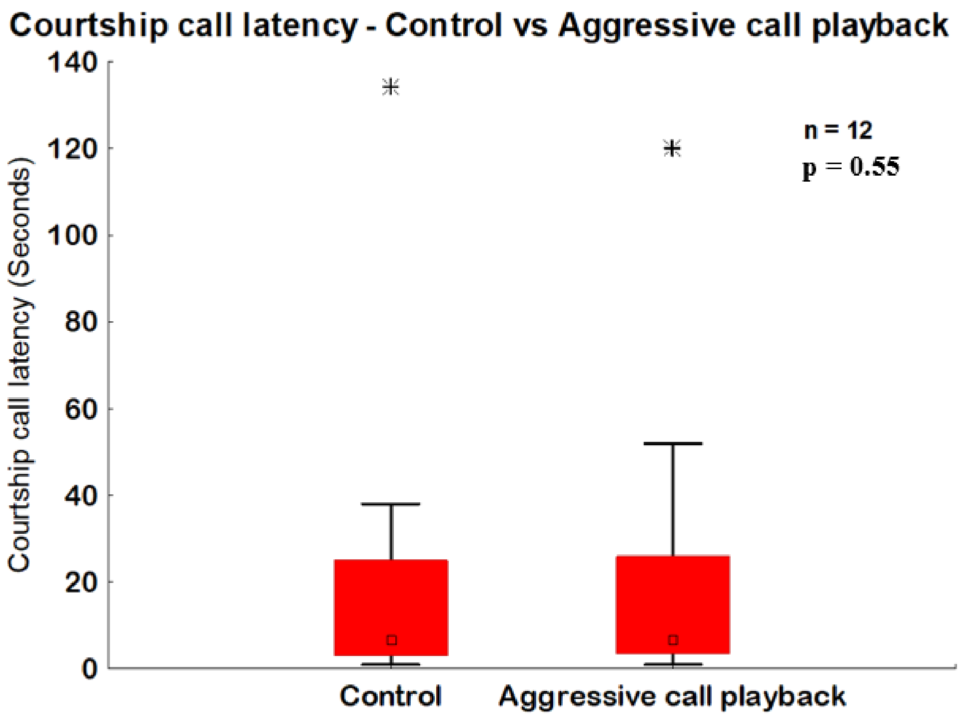
For each analysis, normality test (Shapiro-Wilk test) was performed. If the dataset was normally distributed, paired sample T-test was used. Otherwise, Wilcoxon matched pairs test was performed. Statistica version 10 was used for all statistical analysis.

3.3 Results

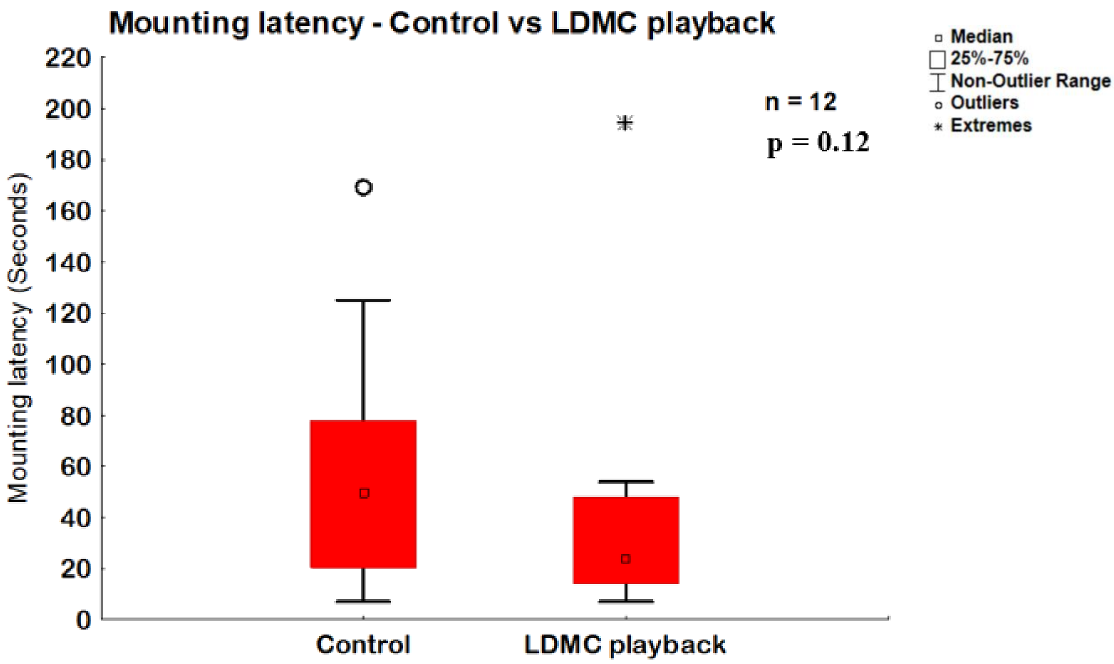
Paired sample T-test and Wilcoxon matched pairs test (significant at $p < 0.05$) were performed according to the normality of dataset. Of all the parameters analysed between different treatments (Silent control, LDMC playback and aggressive playback), no significant differences were observed. Different parameters analyzed are given below:



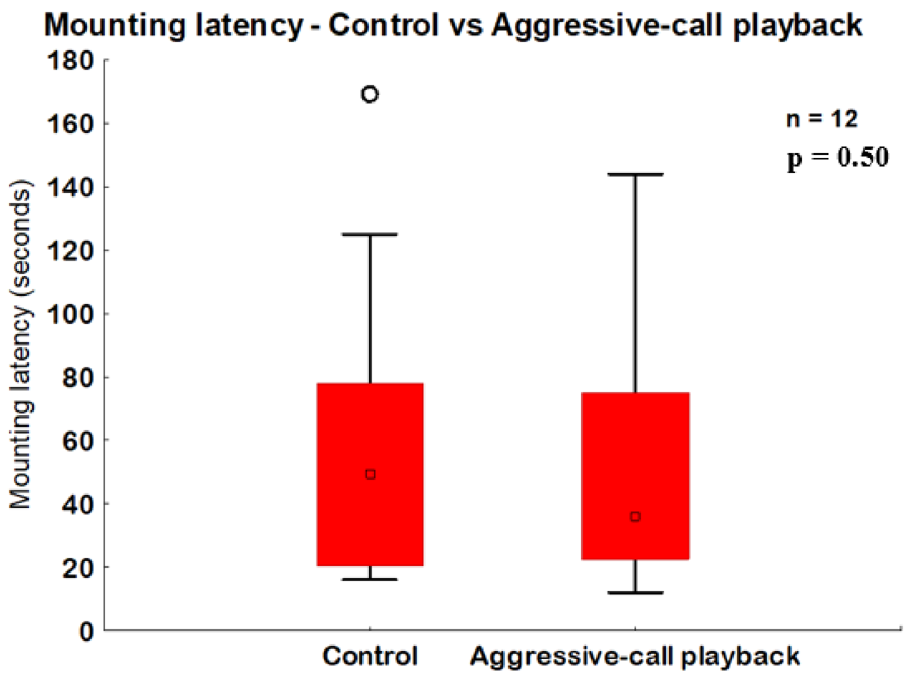
(a)



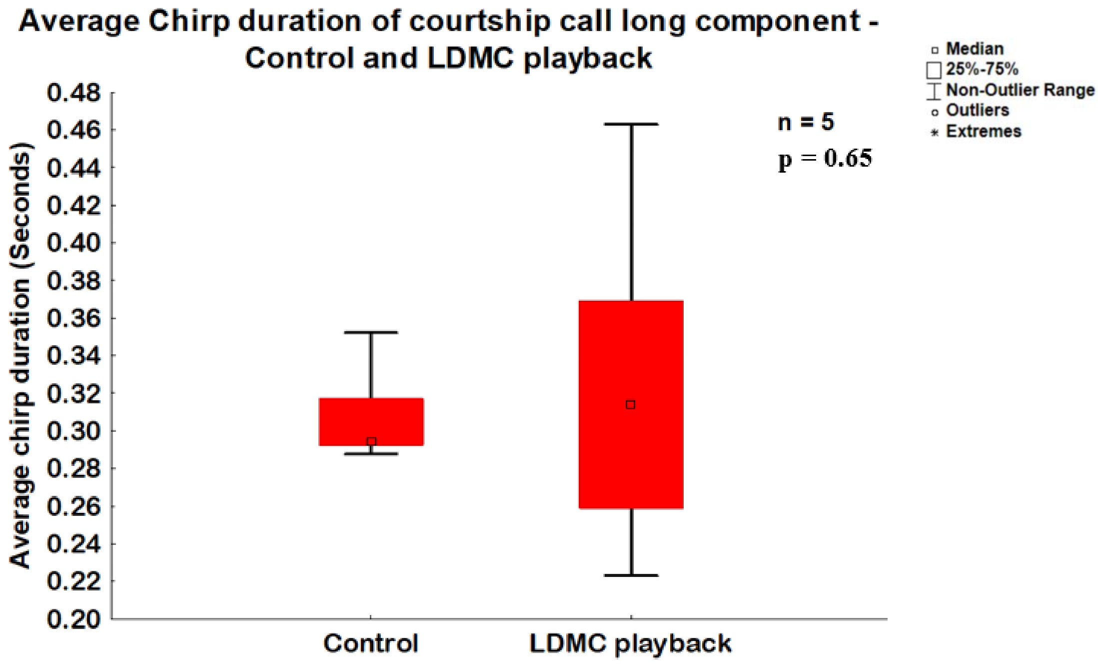
(b)


Mounting latency - Control vs LDMC playback
Median
25%-75%
Non-Outlier Range
Outliers
Extremes
n = 12
p = 0.12
Mounting latency (Seconds)
0
20
40
60
80
100
120
140
160
180
200
220
Control
LDMC playback

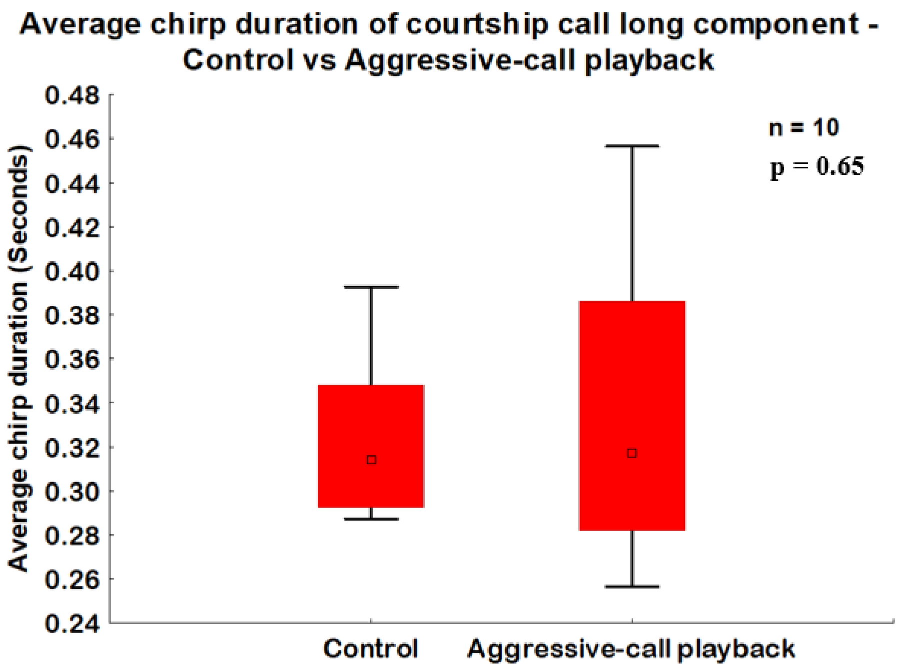
(c)


Mounting latency - Control vs Aggressive-call playback
n = 12
p = 0.50
Mounting latency (seconds)
0
20
40
60
80
100
120
140
160
180
Control
Aggressive-call playback

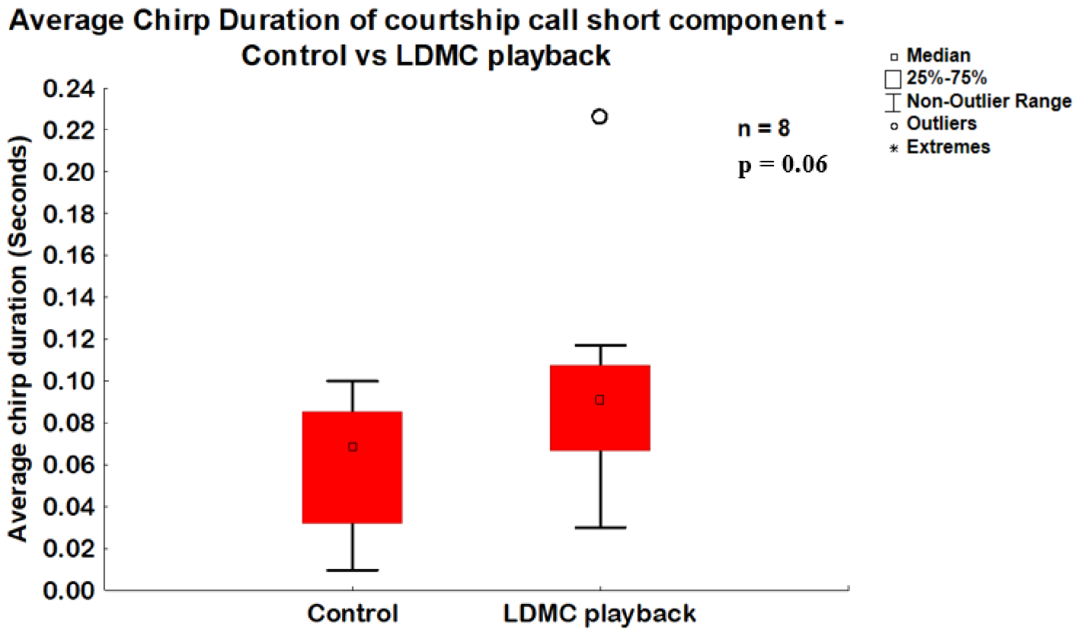
(d)


Average Chirp duration of courtship call long component -
Control and LDMC playback
Median
25%-75%
Non-Outlier Range
Outliers
Extremes
n = 5
p = 0.65
Average chirp duration (Seconds)
0.48
0.46
0.44
0.42
0.40
0.38
0.36
0.34
0.32
0.30
0.28
0.26
0.24
0.22
0.20
Control
LDMC playback

(e)

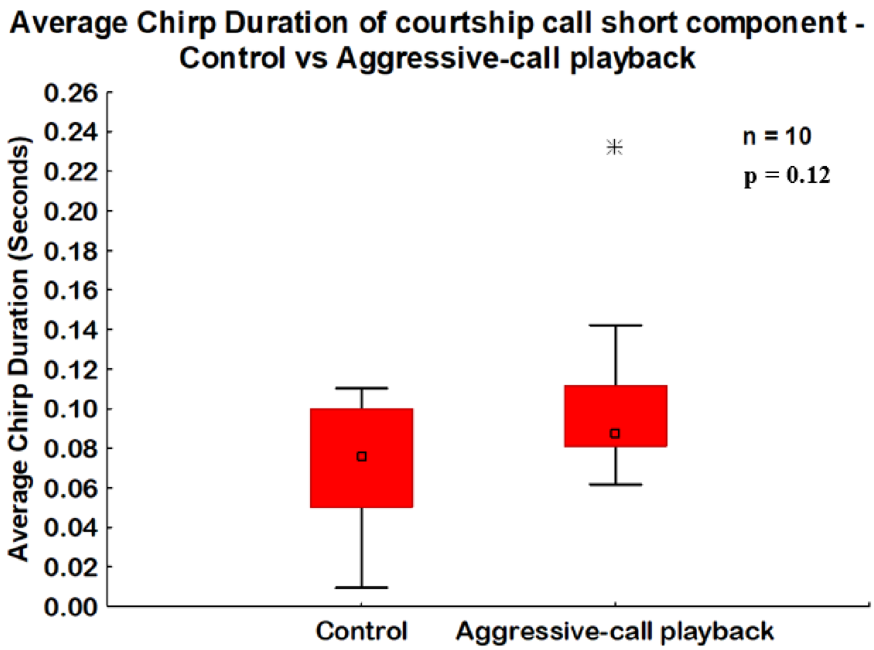

Average chirp duration of courtship call long component -
Control vs Aggressive-call playback
n = 10
p = 0.65
Average chirp duration (Seconds)
0.48
0.46
0.44
0.42
0.40
0.38
0.36
0.34
0.32
0.30
0.28
0.26
0.24
Control
Aggressive-call playback

(f)



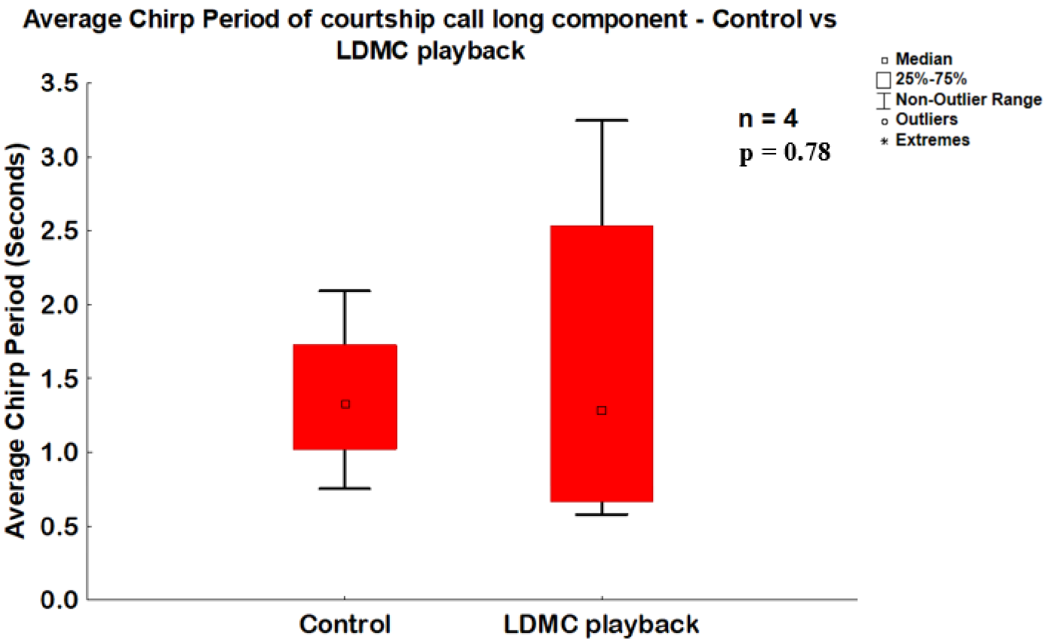
Average Chirp Duration of courtship call short component - Control vs LDMC playback
Median
25%-75%
Non-Outlier Range
Outliers
Extremes
n = 8
p = 0.06
Average chirp duration (Seconds)
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Control
LDMC playback

(g)

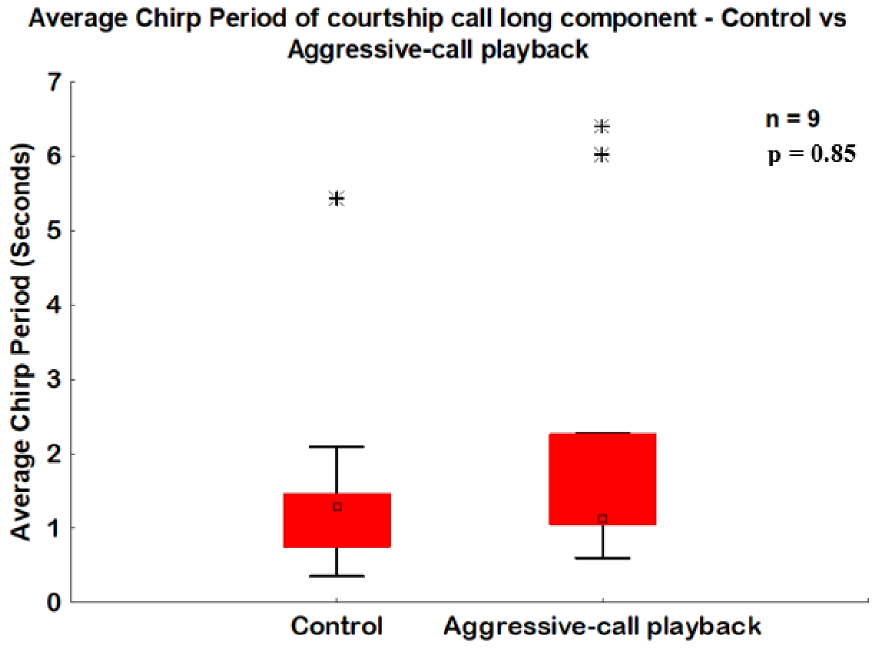


Average Chirp Duration of courtship call short component - Control vs Aggressive-call playback
n = 10
p = 0.12
Average Chirp Duration (Seconds)
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Control
Aggressive-call playback

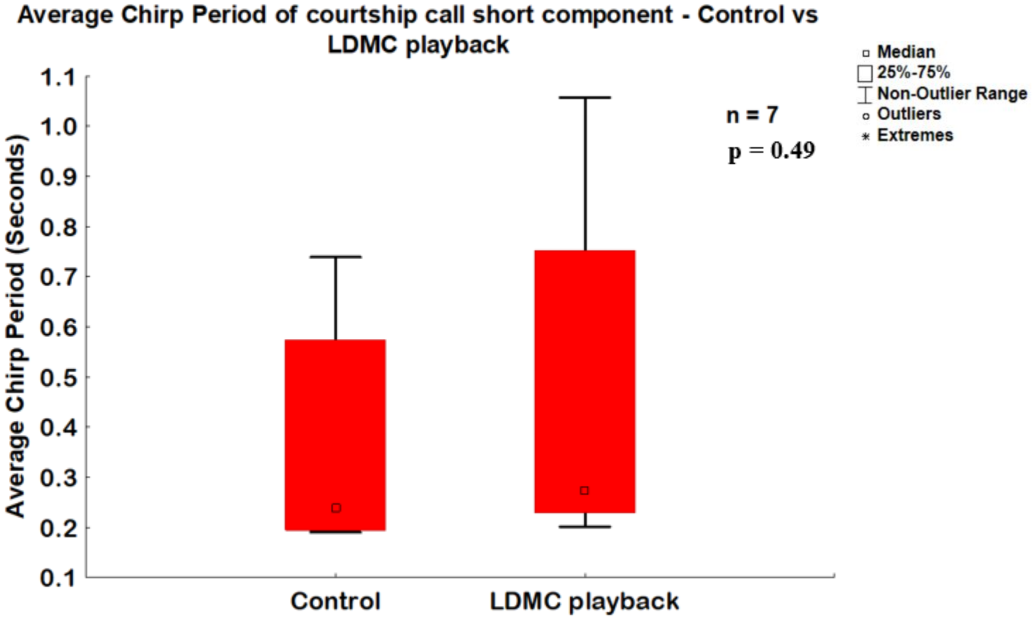
(h)


Average Chirp Period of courtship call long component - Control vs LDMC playback
Median
25%-75%
Non-Outlier Range
Outliers
Extremes
n = 4
p = 0.78
Average Chirp Period (Seconds)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Control
LDMC playback

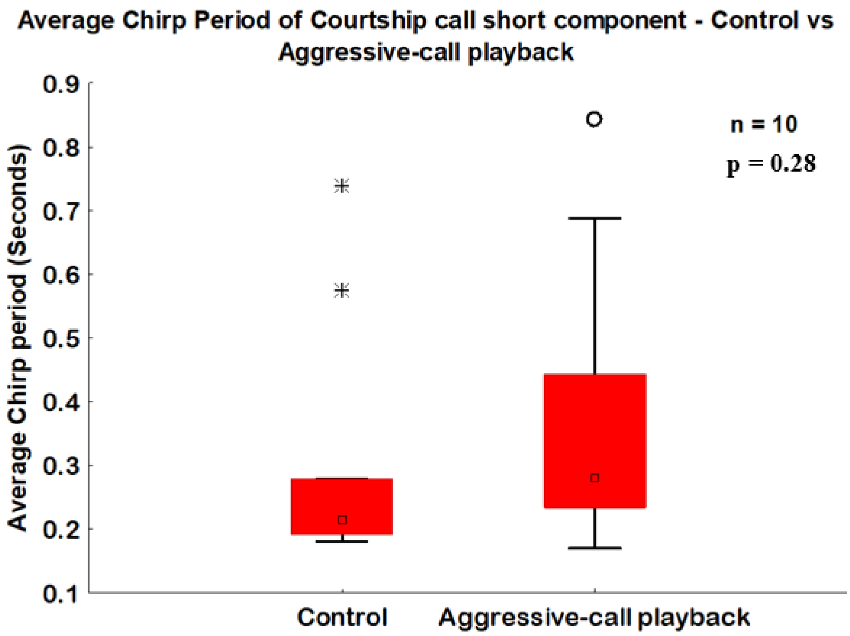
(i)


Average Chirp Period of courtship call long component - Control vs Aggressive-call playback
n = 9
p = 0.85
Average Chirp Period (Seconds)
7
6
5
4
3
2
1
0
Control
Aggressive-call playback

(j)



(k)



(l)

Figure 3.3 Box plots – (a), (b) courtship call latency: control vs LDMC and control vs Aggressive call; (c), (d) Mounting latency: control vs LDMC and control vs aggressive call; (e), (f) Average chirp duration of CC long component: control vs LDMC and control vs aggressive call; (g), (h) Average chirp duration of CC short component: control vs LDMC and control vs aggressive call; (i),(j) Average chirp period of CC long component; control vs LDMC vs control vs Aggressive call; (k),(l) Average chirp period of CC short component: control vs LDMC and control vs aggressive call.

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Chapter 4: To investigate the role of social environment on courtship behaviour of *A. asiaticus*

4.1 Background

Previous studies on 'audience effect' in crickets reveal conflicting results (Tachon *et al*., 1999; Fitzsimmons and Bertram 2013; Montroy *et al.,* 2016 and Judge *et al.* 2010). Most of these studies only looked upon the effect of an audience on the aggressive interactions between the males. The presence of audience effect on mating behaviour of crickets is scarcely looked upon. Sadowski *et al.,* 2002 observed that male courtship behaviour of striped ground cricket changed according to the social environment.

Here, I examine the possible effects of the presence and type of an audience on the courtship behaviour of our study species.

4.2 Methodology

4.2.1 Collection and housings of crickets:

Subadult crickets were collected from the IISER Mohali campus during May – July 2019. Sub adults were kept in a common culture box until they moulted into adults. After final moult, adults were transferred into separate plastic boxes (to ensure no interaction between each other) containing dog food and wet cotton balls for food and nourishment. The temperature was maintained at 24°C. One week prior to the trials, female crickets were separately kept to provide acoustic isolation.

4.2.2 Staging courtship rituals

All the individuals were weighed before the experiment. Courtship pairs were not size-matched. A total of 20 male-female pairs were used for the experiment. Only naïve males and females were used. Females were acoustically isolated at least one week prior to the day of the experiment. Three different treatments - no audience, male audience, and female audience - were given to each pair. The same pair were given at the least 48 hours rest between two treatments in order to mitigate any effects of olfactory learning. The order of treatments given to each pair was randomized with the help of a MATLAB (2019) random number matrix generator code.

Courtship trials were staged inside a circular PVC container (height: 10cm, diameter:15cm) with the base covered with soil. The audience individual was kept inside a perforated, circular, plastic container (height: 8cm, diameter: 4cm), which was kept concentric to the outer PVC container. Perforated plastic container facilitated the transmission of both visual and olfactory cues between the audience and focal animals. A removable cardboard partition separated two equal areas between the outer PVC and inner plastic containers, where the focal animals were introduced.

At the onset of the experiment, focal male-female pair was introduced on either side of the partition, and the respective treatment (no audience control, male audience, or female audience) was introduced inside the middle container. Both the pair and audience were given two minutes acclimatization period before removing the partition. This acclimatization period was given to familiarize the novel environment to test animals and to increase the exposure of audience to the focal pair. Just before the initiation of a mating trial, the audio and video recorders were turned on simultaneously. After removing the partition, male and female crickets are allowed to interact for 5 minutes. The courtship

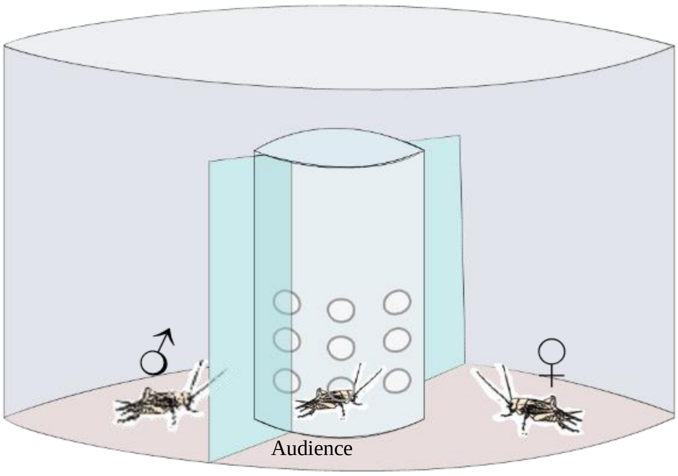


Figure 4.1. Schematic representation of the courtship arena

ritual was declared to be concluded when the female finally mounted the male and the latter initiated spermatophore transfer. Focal pair was separated right before the transfer of spermatophore has taken place. Before each treatment, the entire arena was cleaned using 70% ethanol, and soil was shuffled to make sure the elimination of any odour cues.

4.2.3 Acoustic and camera recordings

To record one complete experimental session (starts from the removal of partition till the mounting), CANON XA20 Infrared camera (Canon corp., Japan) mounted on a tripod was used. TASCAM DR-40 linear PCM recorder (DR-07 Mk II, TEAC Professional, USA) mounted on a tripod was used for an acoustic recording of courtship calls.

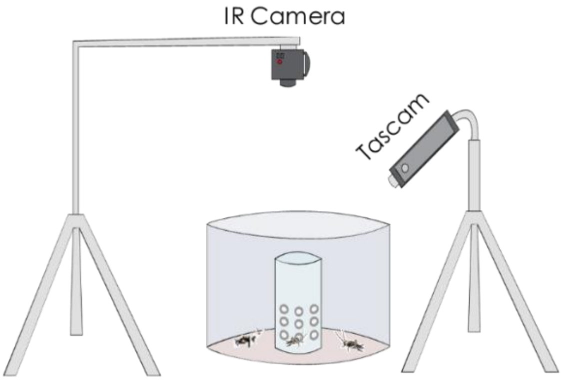


Figure 4.2. Schematic representation of the experimental setup

4.2.4 Data analysis

All the video recordings were analysed using Shotcut Video Editor. All the audio recordings were first high-pass (>2000Hz) filtered and then amplified using Audacity 2.3.1 . Later, Raven Pro 1.5.0 (Cornell Laboratory of Ornithology,Ithaca, NY) as used to analyse various temporal and spectral parameters of the call.

4.2.5 Analysis of behaviour and acoustics

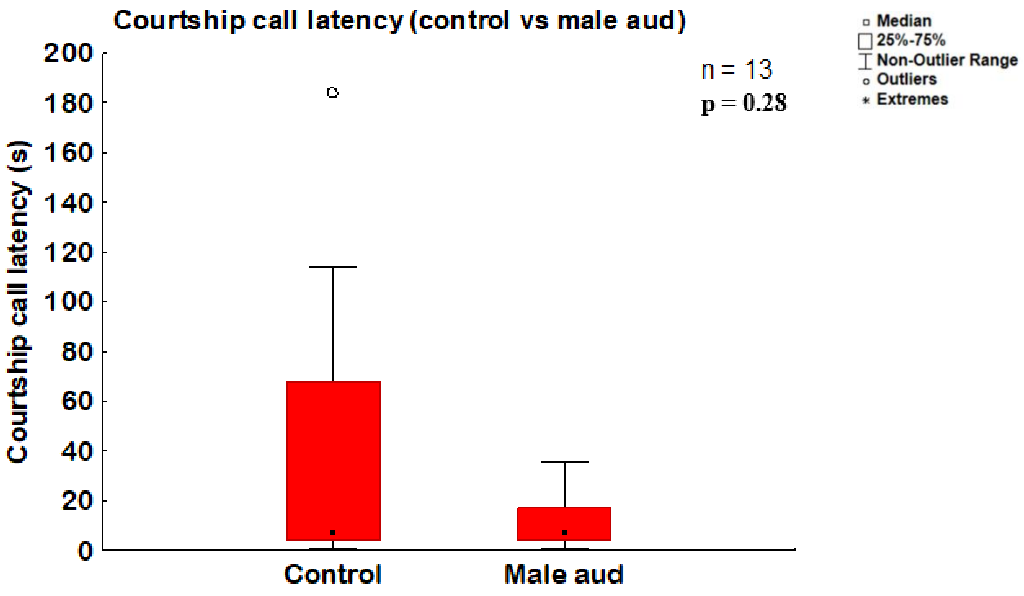
All the behavioural and acoustic parameters described in chapter 3 section 3.2.6 was analysed.

4.2.6 Statistical analysis

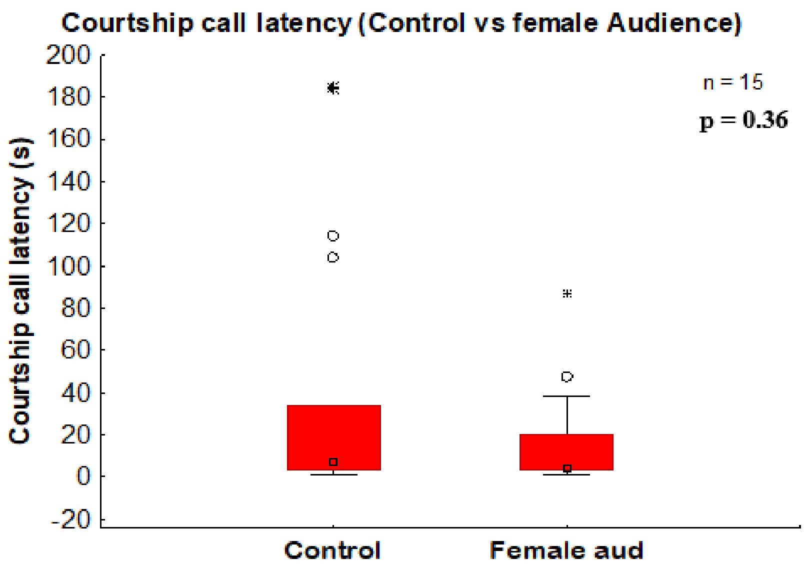
For each analysis, normality test (Shapiro-Wilk test) was performed. If the dataset was normally distributed, paired sample T-test was used. Otherwise, Wilcoxon matched pairs test was performed. Statistica version 10 was used for all statistical analysis.

4.3 Results

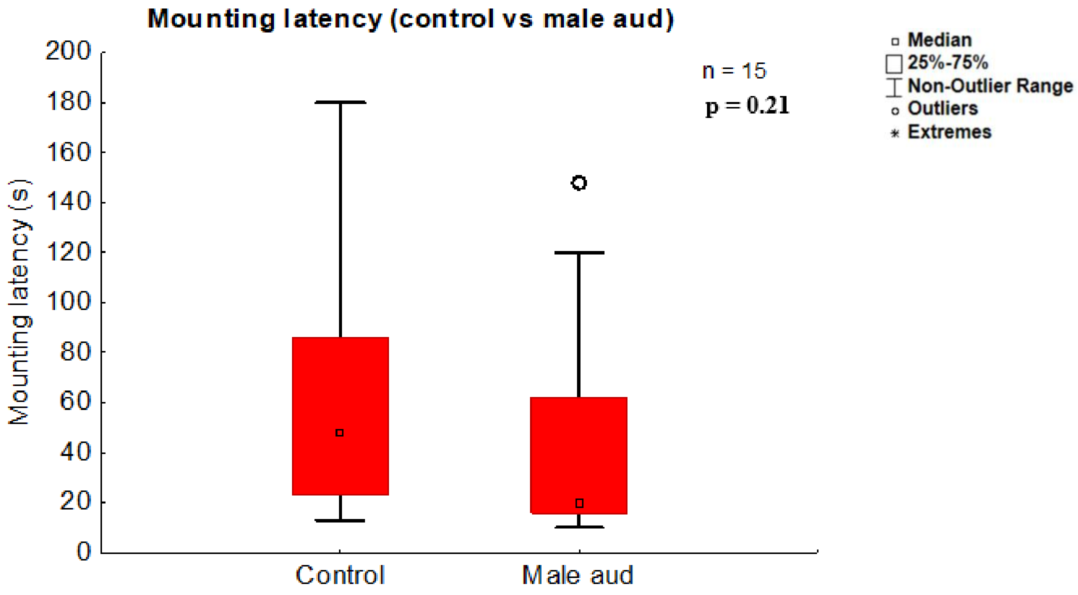
Paired sample T-test and Wilcoxon matched pairs test (significant at $p < 0.05$) were performed according to the normality of dataset. Of all the parameters analysed between different treatments (no audience control, male audience and female audience), no significant differences were observed. Different parameters analyzed are given below:



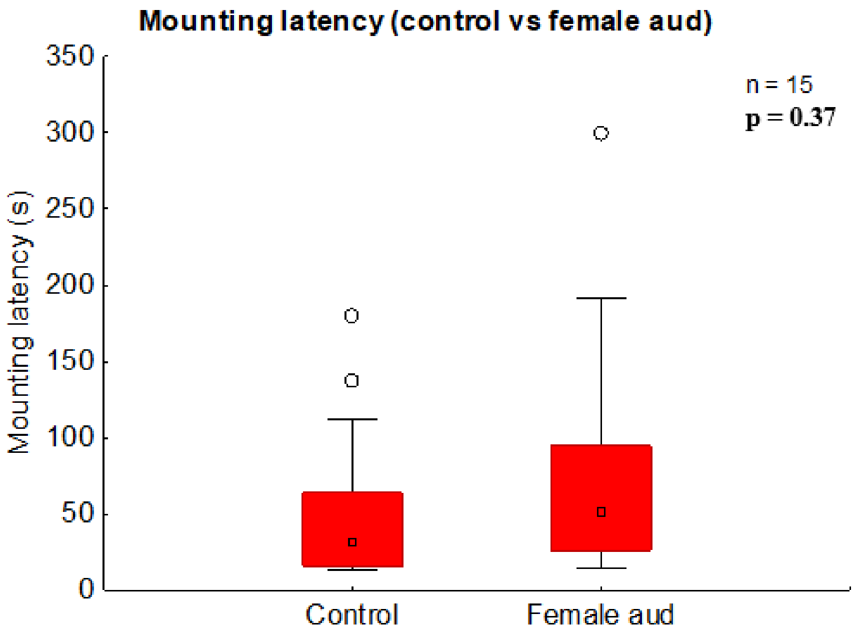
(a)



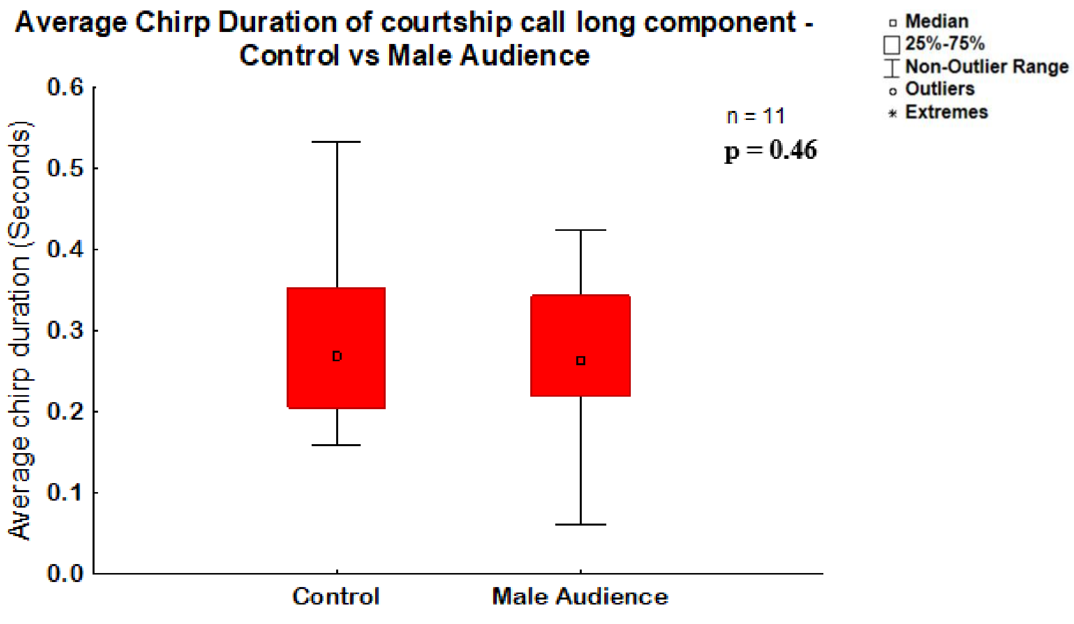
(b)


Mounting latency (control vs male aud)
Median
25%-75%
Non-Outlier Range
Outliers
Extremes
n = 15
p = 0.21
Mounting latency (s)
200
180
160
140
120
100
80
60
40
20
0
Control
Male aud

(c)

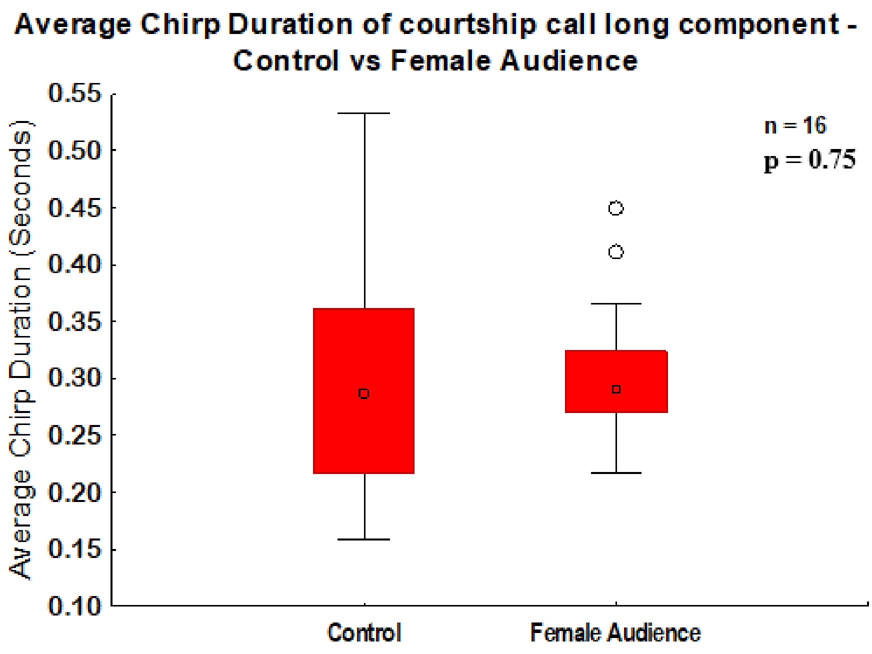

Mounting latency (control vs female aud)
n = 15
p = 0.37
Mounting latency (s)
350
300
250
200
150
100
50
0
Control
Female aud

(d)



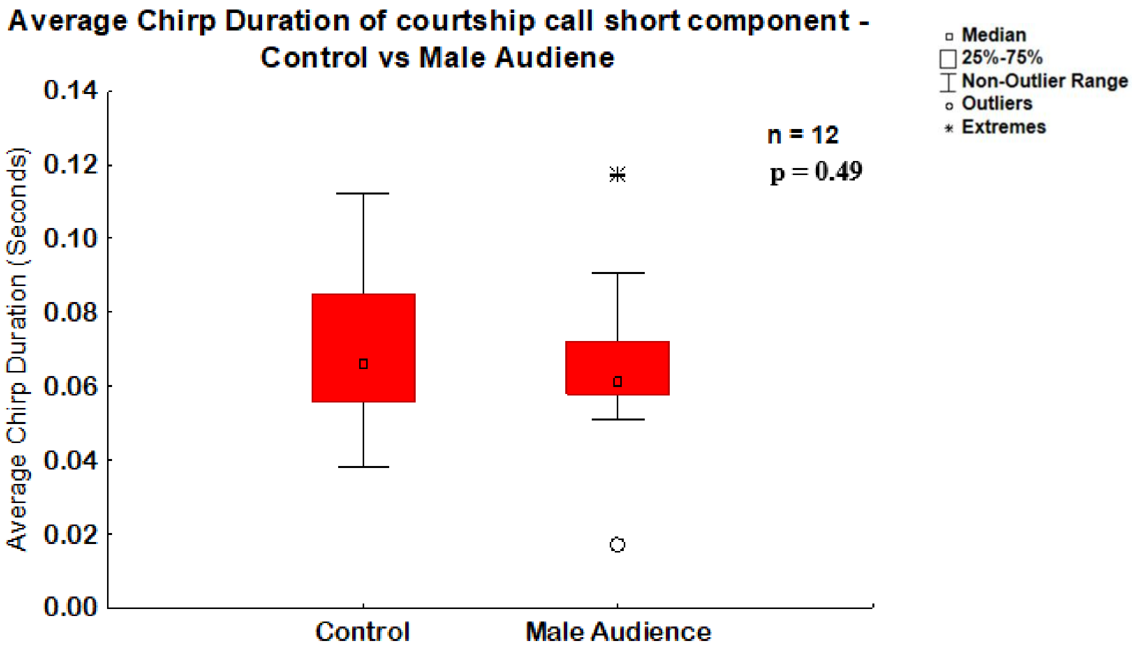
Average Chirp Duration of courtship call long component -
Control vs Male Audience
Median
25%-75%
Non-Outlier Range
Outliers
Extremes
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Average chirp duration (Seconds)
n = 11
p = 0.46
Control
Male Audience

(e)

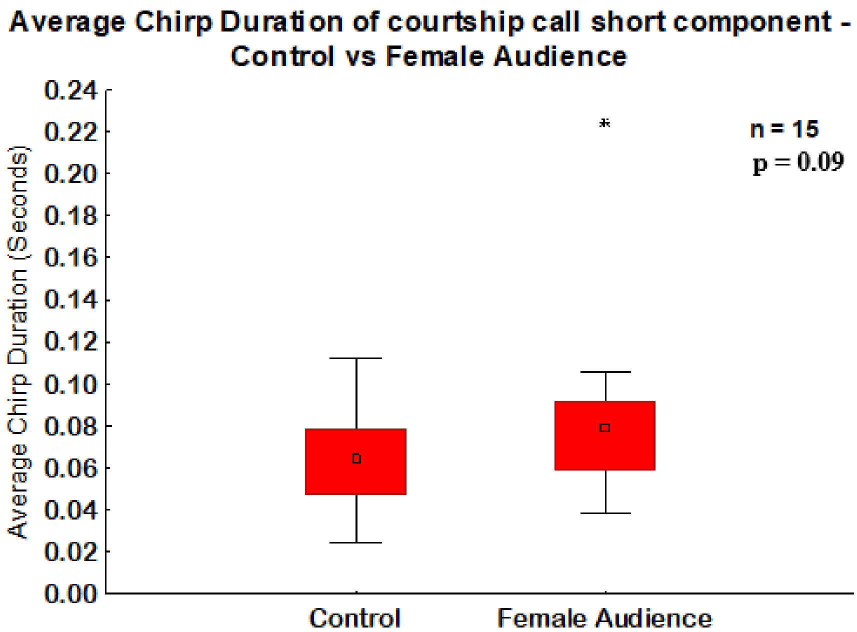


Average Chirp Duration of courtship call long component -
Control vs Female Audience
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
Average Chirp Duration (Seconds)
n = 16
p = 0.75
Control
Female Audience

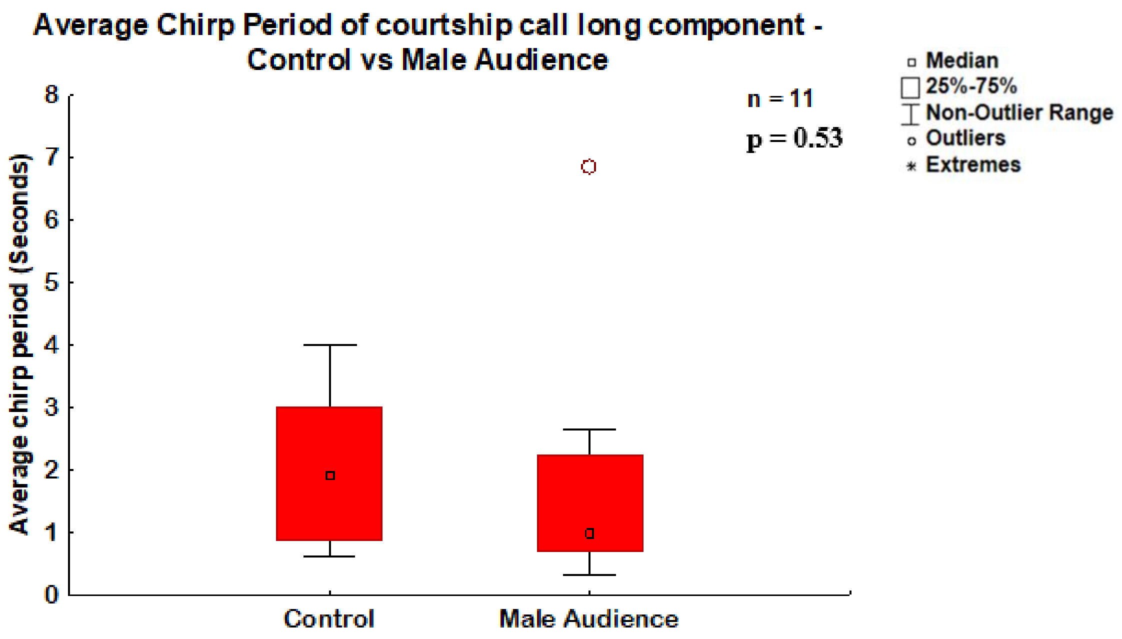
(f)


Average Chirp Duration of courtship call short component -
Control vs Male Audiene
Median
25%-75%
Non-Outlier Range
Outliers
Extremes
n = 12
p = 0.49
Average Chirp Duration (Seconds)
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Control
Male Audience

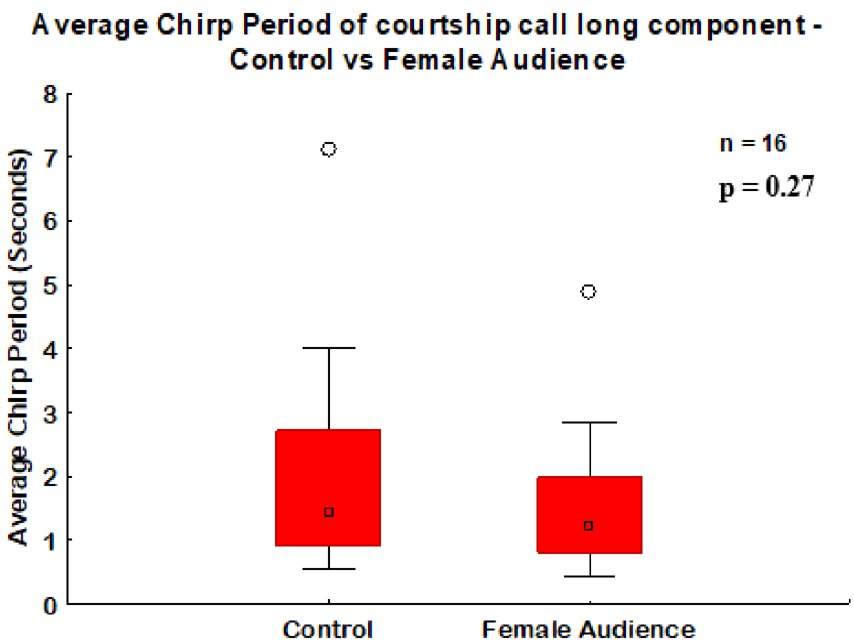
(g)


Average Chirp Duration of courtship call short component -
Control vs Female Audience
n = 15
p = 0.09
Average Chirp Duration (Seconds)
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Control
Female Audience

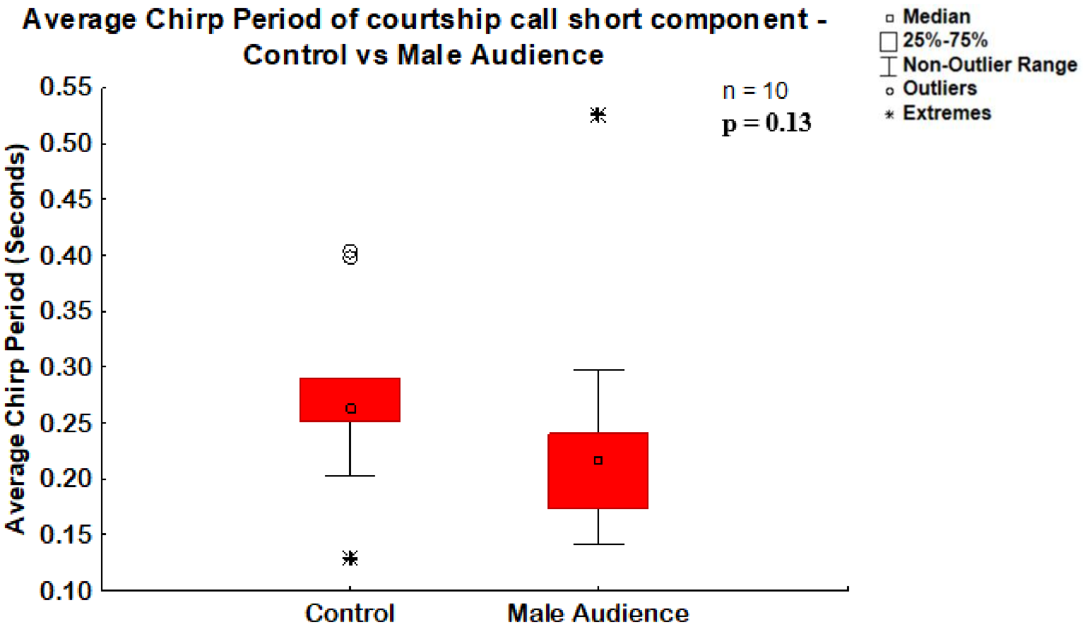
(h)


Average Chirp Period of courtship call long component -
Control vs Male Audience
Median
25%-75%
Non-Outlier Range
Outliers
Extremes
n = 11
p = 0.53
Average chirp period (Seconds)
8
7
6
5
4
3
2
1
0
Control
Male Audience

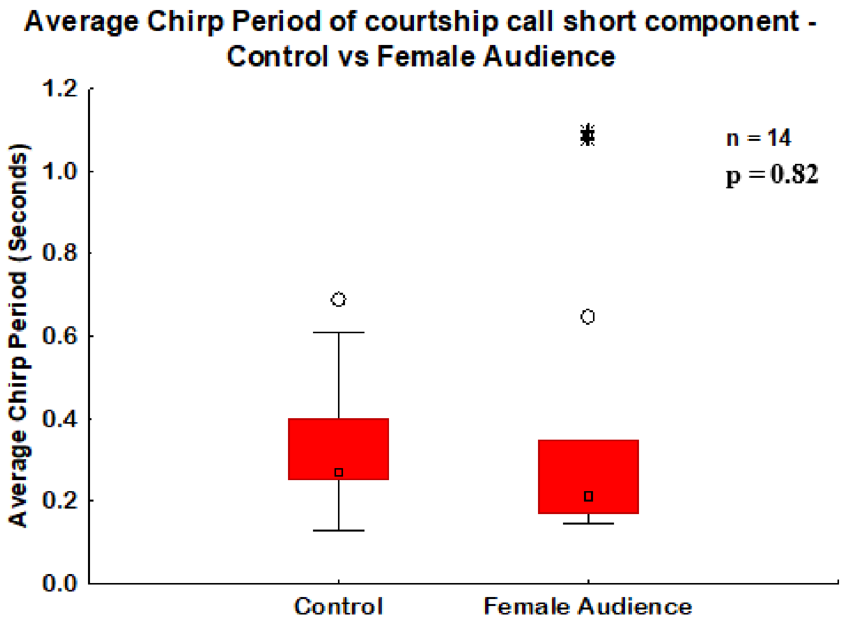
(i)


Average Chirp Period of courtship call long component -
Control vs Female Audience
n = 16
p = 0.27
Average Chirp Period (Seconds)
8
7
6
5
4
3
2
1
0
Control
Female Audience

(j)



(k)



(l)

Figure 4.3. (a),(b) CC latency: control vs male audience and control vs female audience; (c),(d) Mounting latency: control vs male audience and control vs female audience; (e),(f) Average chirp duration of CC long component: control vs male audience and control vs female audience; (g),(h) Average chirp duration of CC short component: control vs male audience and control vs female audience; (i),(j) Average chirp period of CC long component: Control vs male audience and control vs female audience; (k),(l) Average chirp period of CC short component: Control vs male audience and control vs female audience

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Chapter 5: Discussion and future direction

5.1 Discussion

Published studies of audience effect on cricket aggression reveal conflicting results. Some species like *Gryllus bimaculatus, Gryllus veletis* and *Gryllus assimilis* are more aggressive in the presence of an audience (Tachon *el al.*, 1999; Fitzsimmons and Betram, 2013; Montroy and Loranger, 2016) whereas *Gryllus pennsylvanicus* shows no evidence of aggressive escalation in the presence of an audience (Judge, 2010). Studies examining the effect of social environment on the courtship behaviour of crickets are still fewer, to the best of my knowledge. One study on *Allonemobius socius* observed a change in both male courtship signaling behaviour as well as female evaluation of the males when an audience was present (Sadowski, 2002).

My first objective was to quantify the courtship behaviour and the courtship call of *A. asiatiucs.* Second objective was to investigate whether the acoustic proxy for the presence of an audience or the actual presence of an audience elicits any kind of change in the courtship or acoustic behaviour of *A. asiaticus*. Two different acoustic signals – LDMC and aggressive song – were used as a proxy for the presence of audience nearby. Courtship song was not used as an acoustic proxy considering its subtle and private nature: chances of encountering courtship call from an audience in wild for a mating pair is low. For the next part of my experiment, courtship trials of focal pairs were carried out in the apparent presence of either a male or a female audience. Since a male audience can be detected as a potential rival and a potential mate by the male and female undergoing courtship respectively, I expected an alteration in the duration or the intensity of some courtship behaviours.

Major findings of my study are:

a) Courtship ritual of *A. asiaticus* consists of three different and distinct stages – (i) antennation until courtship song initiation, (ii) courtship song initiation till mounting and (iii) mounting until spermatophore transfer.
b) Different kinds of acoustic proxy for the presence of a male audience nearby elicits no significant changes in the courtship behaviour of the focal mating pair.
c) The presence of any kind of audience elicits no significant change in the courtship behaviour of the focal mating pair.

During both audience effect experiments, the presence of an additional individual or an acoustic proxy for the same elicited no significant change in the courtship and acoustic behaviour of the focal mating pair. One possible reason for this observation could be the spacing of *A. asiaticus* in the field. Habitat spacing studies on *A. asiaticus* from our lab indicate that the average nearest neighbor distance of male – female and male – male pair are 2.1 meter and 3.5 meter respectively (Singh R, Phd thesis, 2020). This spacing in turn indicates a reduced chance of physical interactions as well as the presence of an audience during courtship interactions of *A. asiaticus.* Another possible reason for observing no change of behaviour in the presence of an audience can be attributed to the robustness of courtship behaviour itself. Courtship experiments in *G. bimaculatus* have shown that social context and sexual experience has little effect on the courtship behaviour than it has on agonistic behaviour (Adamo and Hoy, 1993). The same study observed that mating opportunities are exploited by sexually receptive crickets, irrespective of their social experience or other external factors, suggesting courtship behaviour is a robust and innate behavioural phenomenon. So, once initiated by antennation, individuals undergoing courtship behaviour may continue until mounting without paying much heed to the audiences present nearby.

Usually, communication via private channels, (such as courtship signaling in crickets) that are directed to a potential mate, use sensory channels not available to the audience (Matos and Schlupp, 2005). True dyadic interactions may be of short duration and limited to signals transmitted in close proximity like chemosensory signals in crickets which cannot be detected by an audience present in near vicinity. This could be another reason for not observing proper audience effect in my study. The striped ground cricket (*Allonemobius socius*) in which audience effect on courtship behaviour has been observed has a ‘complex’ courtship ritual with variety of male behaviours (three types of songs, two types of nuptial gifts) and also requires female responses to these behaviours for successful copulation to occur (Mays, 1971). Compared to the relatively simple courtship rituals of other gryllid species, the complex courtship ritual incorporating multiple components may give more opportunities for *A. soicus* to alter their courtship signaling in the presence of an audience.

A previous study carried out in our lab (Kalra L, Master's thesis, 2017) on the effect of an audience in agonistic interactions of *A. asiaticus* also observed no significant audience effect on aggressive behaviour. Thus, observations from my experiments and previous experiments from our lab seem to suggest that there is no audience effect in the context of courtship and agonistic interaction between individuals in *A.asiaticus.*

5.2 Future directions

How broadly or narrowly we wish to define the social context of signaling is a recurring question in the general context of communication networks occurs. Dyadic interactions (with a very few exceptions) are in fact implanted in a broad social background or network. Often this social aspect of signaling is not taken into consideration while studying animal communication.

Observations from my experiments and previous experiments performed in our lab indicate that there is no audience effect in the context of agonistic and courtship interactions in *A. asiaticus.* However, how the presence of an audience influences post-copulatory behaviour still remains to be examined. *A. asiaticus* displays a characteristic post-copulatory mate guarding behaviour and how this behaviour changes with varying social environments will be an interesting topic for investigation.

Two different studies on audience effect from our lab used entirely different setups of presenting the audience. Maybe a more sophisticated arena with better audience presentation capability can observe something we might have missed. Further, audiences and focal individuals I used were randomly paired. A host of additional factors like size, status, age, and social experience of the audience may affect the signaling behaviour of focal individuals. Follow up investigations should focus on these factors.

'Bystander effect' is another phenomenon that needs to be investigated but often ignored in the context of cricket behaviour. Bystander effect is when an individual observing a signaling interaction changes its estimation of the abilities of a signaler based on what it observes (Dugatkin, 2001). Bystanders incorporate this extracted information for use in future encounters with the observed individual. Studies on Green swordtail fish (*Xiphophorus helleri)* and Guppy (*Poecilia reticulata)* indicate the presence of bystander effect in the context of both agonistic interactions and female mate choice, respectively (Earley, Tinsley and Dugatkin, 2003; Dugatkin, 1992). Handful of studies that have looked into the effect of social environment in cricket behaviour focused on audience effect and neglected the potential existence of bystander effect. Since agonistic and courtship interactions in crickets are acoustically active, a dyadic interaction having acoustic components will be transmitted over greater distance, creating potential bystanders. Given such interactions are common and energetically expensive in field crickets, a bystander may benefit from observing such interactions. So, the utility of extracting information and storing it for subsequent retrieval by the bystander needs to be examined.

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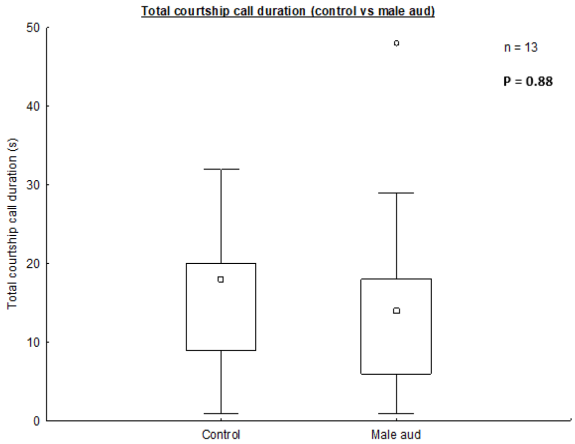
Appendix

Rest of the parameters analysed in chapter 4: To investigate the effect of social environment on courtship behaviour of *A. asiaticus.*



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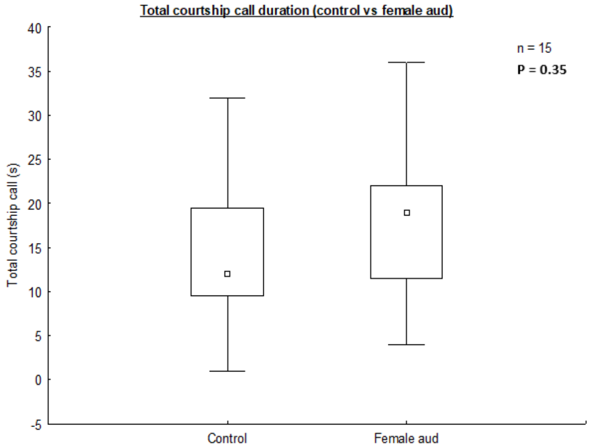

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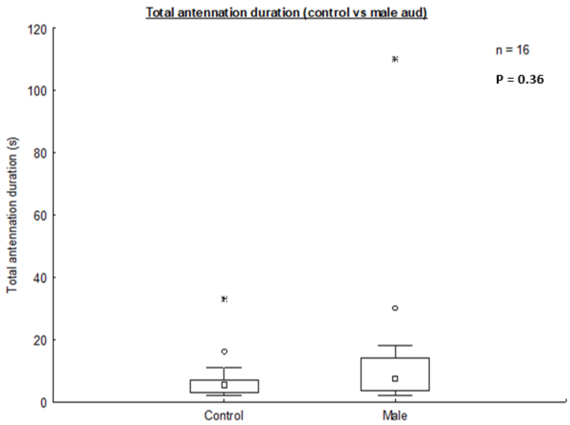

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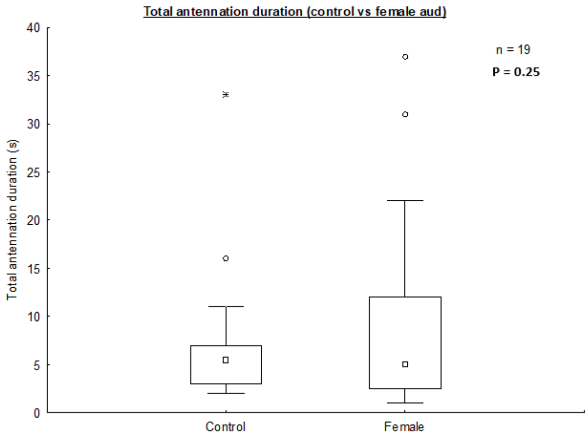

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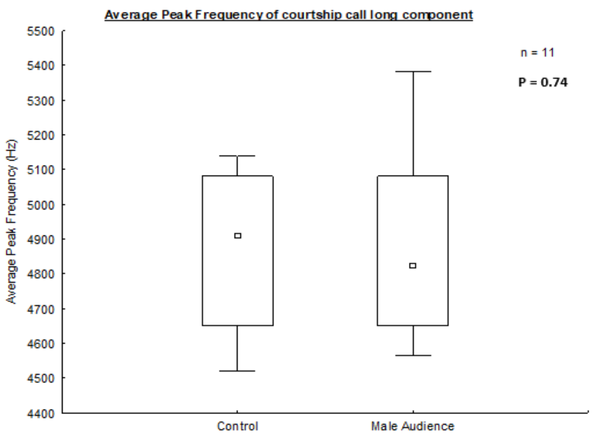

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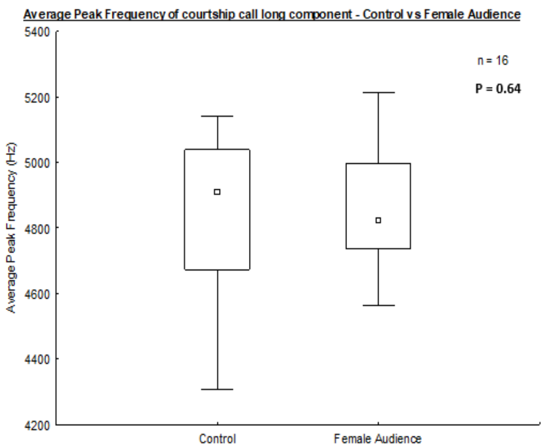

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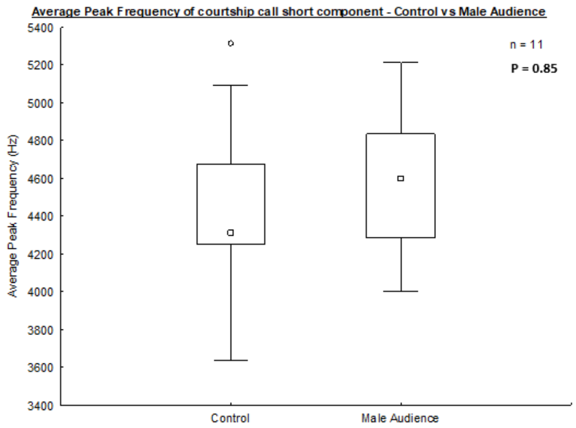

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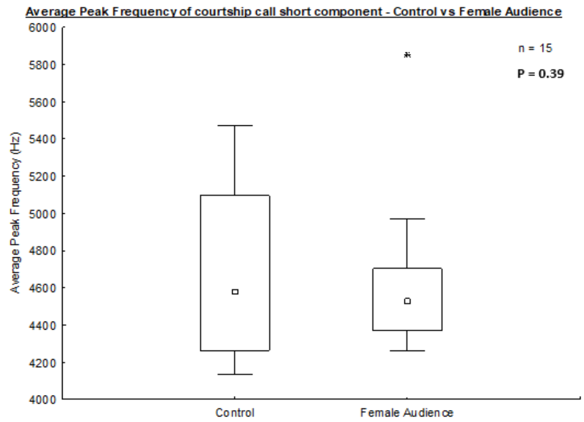
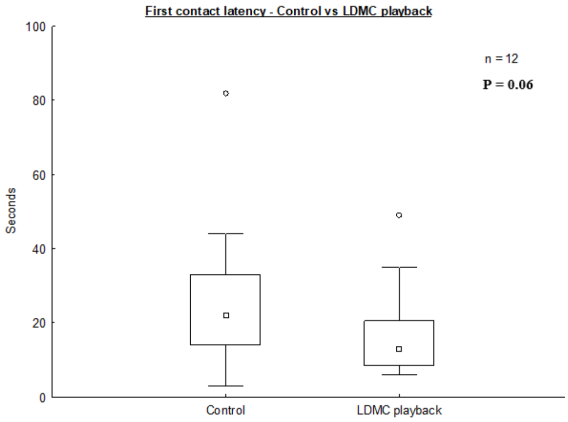


(h)

Figure. Box plots: (a),(b) Total courtship call duration: control vs male audience and control vs female audience; (c),(d) Total antennation duration: control vs male audience and control vs female audience; (e), (f) Average peak frequency of CC long component: control vs male audience and control vs female audience; (g), (h) Average peak frequency of CC short component: control vs male audience and control vs female audience

Rest of the parameters analysed in chapter 3: To investigate the role of acoustic environment on courtship behaviour of *A. asiaticus*.



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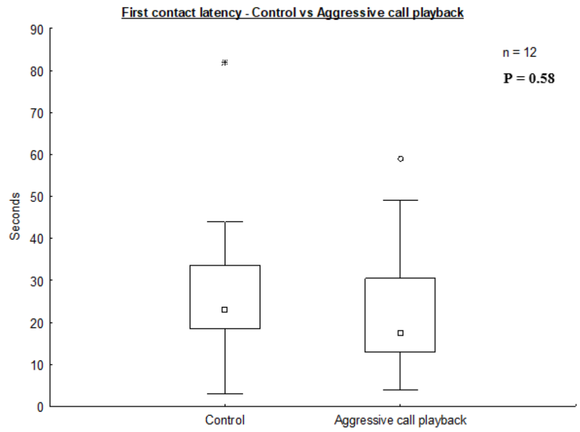

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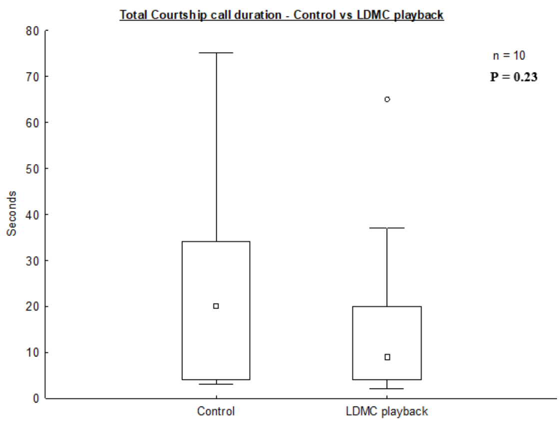

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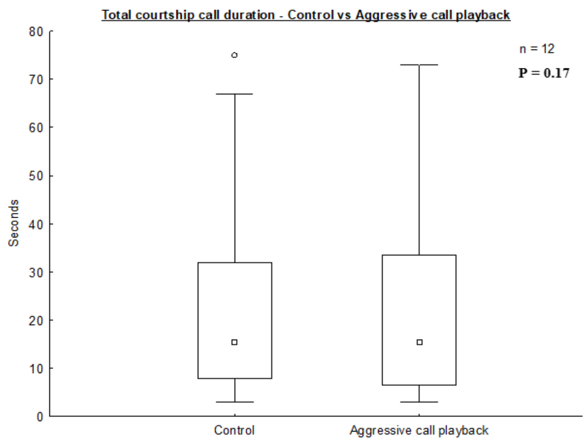

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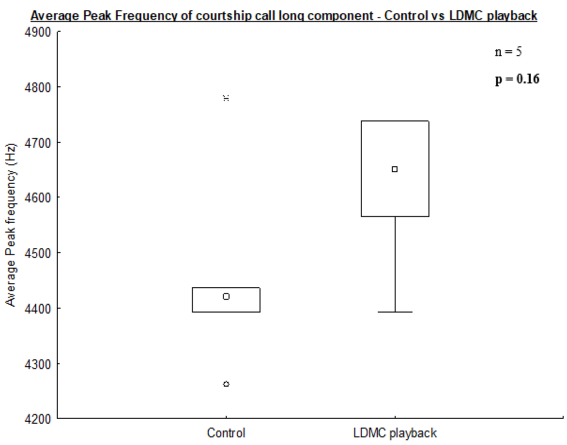

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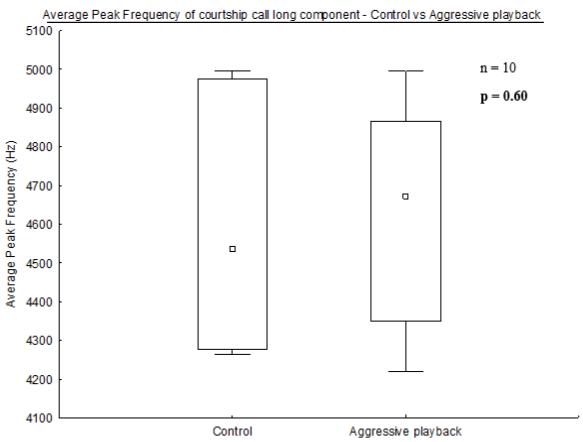

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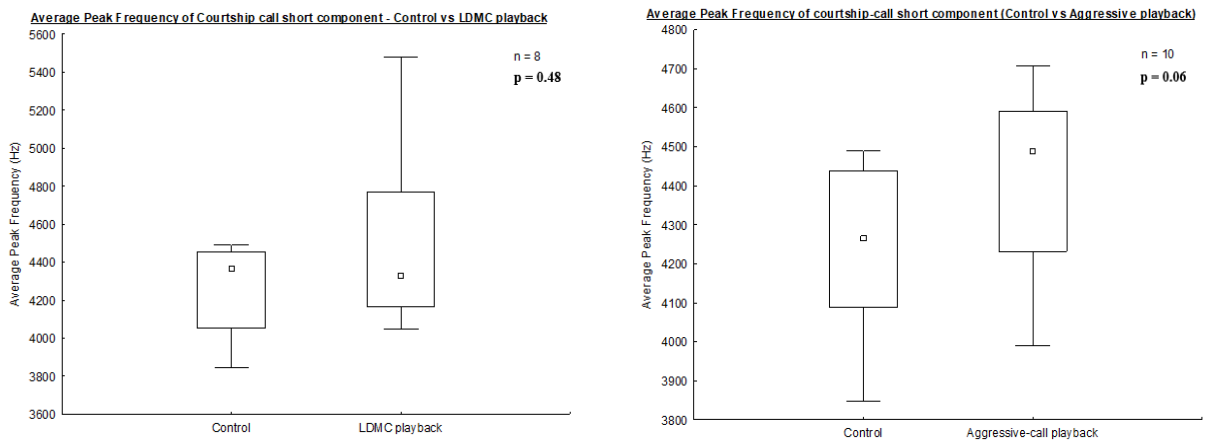

(g) (h)

Figure. Box plots: (a),(b) First contact latency: Control vs LDMC and control vs aggressive call playback; (c),(d) Total courtship call duration: control vs LDMC and control vs aggressive call playback; (e),(f) Average peak frequency of CC long component: control vs LDMC and control vs aggressive call playback; (g),(h) Average peak frequency of CC short component: control vs LDMC and control vs aggressive call playback.